Characterization of nisin F and its role in the control of respiratory tract and skin infections

by

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

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Summary

Multidrug resistant strains of *Staphylococcus aureus* is presenting an increasing threat, especially immune compromised individuals. Many of these strains have developed resistance to newly approved drugs such as quinupristin-dalfopristin, linezolid and daptomycin. The search for alternative treatment, including bacteriocins (ribosomally synthesized antimicrobial peptides) of lactic acid bacteria is increasing .

Lactococcus lactis subsp. *lactis* F10, isolated from freshwater catfish, produced a new nisin variant active against clinical strains of *S. aureus*. The operon encoding nisin F is located on a plasmid and the structural gene has been sequenced. The lantibiotic is closely related to nisin Z, except at position 30 where valine replaced isoleucine.

The antimicrobial activity of nisin F against *S. aureus* was tested in the respiratory tract of Wistar rats. Non-immunosuppressed and immunosuppressed rats were intranasally infected with *S. aureus* K and then treated with either nisin F or sterile physiological saline. Nisin F protected immunosuppressed rats against *S. aureus*, as symptoms of an infection were only detected in the trachea and lungs of immunosuppressed rats treated with saline. The safety of intranasally administered nisin F was also evaluated and proved to have no adverse side effects.

The potential of nisin F as an antimicrobial agent to treat subcutaneous skin infections was evaluated by infecting C57BL/6 mice with a bioluminescent strain of *S. aureus* (Xen 36). Immunosuppressed mice were treated with either nisin F or sterile physiological saline 24 h and 48 h after infection with subcutaneously injected *S. aureus* Xen 36. Histology and bioluminescence flux measurements revealed that nisin F was ineffective in the treatment of deep dermal staphylococcal infections. Non-infected and infected mice treated with nisin F had an influx of polymorphonuclear cells in the deep stroma of the skin tissue. This suggested that nisin F, when injected subcutaneously, may have modulated the immune system.

Nisin F proved an effective antimicrobial agent against *S. aureus*-related infections in the respiratory tract, but not against subcutaneous infections. The outcome of nisin F treatment thus depends on the route of administration and site of infection.

Opsomming

Multi-antibiotika weerstandbiedende *Staphylococcus aureus* stamme se weerstand teen verskeie antimikrobiese middels hou ‘n groot gevaar in, veral vir persone met ‘n verwronge immuniteit. Baie stamme van *S. aureus* ontwikkel voortdurend weerstand teen nuut-ontwikkelde antimikrobiese middels soos byvoorbeeld quinupristin-dalfopristin, linezolid en daptomycin. Die belangstelling in alternatiewe antimikrobiese middels, onder andere ook bakteriosiene (ribosomaal gesintetiseerde antimikrobiese peptiede), is aan die toeneem.

Lactococcus lactis subsp. *lactis* F10, geïsoleer van ‘n varswater barber, produseer ’n nuwe variasie nisien aktief teen kliniese stamme van *S. aureus*. Die operon wat vir nisien F kodeer is gelokaliseerd op ’n plasmied en die DNA-volgorde van die strukturele geen is bepaal. Nisien F is die naaste verwant aan nisien Z, behalwe vir die voorkoms van valien in plaas van ‘n isoleusien op posisie 30.

Die antimikrobiese aktiwiteit van nisien F teen *S. aureus* is in die respiratoriese lugweë van Wistar rotte getoets. Rotte met ‘n gesonde immuunstelsel en rotte met ‘n onderdrukte immuunstelsel is intranasaal met *S. aureus* K geïnfekteer en daarna met nisien F of ‘n steriele fisiologiese soutoplossing behandel. Nisien F het immuun-onderdrukte rotte beskerm teen *S. aureus* aangesien simptome van ’n infeksie net in die tragea en longe van immuun-onderdrukte rotte wat met ‘n soutoplossing behandel is, waargeneem kon word. Die toksisiteit van intranasaal geadministreerde nisien F is geëvalueer en geen newe-effekte is waargeneem nie.

Die potensiaal van nisien F as ’n antimikrobiese middel om onderhuidse velinfeksies te behandel is ondersoek deur C57BL/6 muise met ’n bio-luminiserende stam van *S. aureus* (Xen 36) te infekteer. Immuun-onderdrukte muise is met nisien F of steriele fisiologiese soutoplossing behandel 24 en 48 h nadat hulle met *S. aureus* Xen 36 onder die huid geïnfekteer is. Histologie en bioluminiserende fluksie-lesings het getoon dat nisien F oneffektief is in die behandeling van diep dermale *S. aureus*-geassosieerde infeksies. Ongeïnfekteerde en geïnfekteerde nisien-behandelde muise het ’n influks van polimorfonukleêre selle in die diep stroma van die velweefsel teweeggebring. Nisien F, onderhuids ingespuit, mag dalk tot modulering van die immuunstelsel aanleiding gee.

Nisin F is effektief teen *S. aureus*-geassosieerde infeksies in lugweë, maar nie teen onderhuidse infeksies nie. Die effektiwiteit van nisin F hang af van die roete van adiministrasie en die area van infeksie.

Preface

The literature study is an overview of *Staphylococcus aureus* and its association with respiratory tract and skin infections. Bacteriocins and their applications in the medical field are also discussed with emphasis on *in vivo* techniques used to study antimicrobial efficiency.

The manuscript "Characterization of the structural gene encoding nisin F, a new lantibiotic produced by *Lactococcus lactis* subsp. *lactis* isolate from freshwater catfish (*Clarias gariepinus*)" has been published in *Applied and Environmental Microbiology* (2008; **74:**547-549).

The manuscript "Nisin F in the treatment of respiratory tract infections caused by *Staphylococcus aureus*" has been published in *Letters in Applied Microbiology* (2009; **48:**65-70).

The chapter "Nisin F in the treatment of subcutaneous skin infections by monitoring bioluminescent *Staphylococcus aureus* non-invasively and in real-time" has been prepared for submission to the *Journal of Antimicrobial Chemotherapy*.

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Contents

Chapter 1

Introduction

Antibiotics, imperative in modern medicine, are at risk of becoming ineffective in combating bacterial infections. The increasing occurrence of antibiotic resistance and the lack of newly discovered antibiotics are the two major factors responsible. The decreased susceptibility of bacteria against antibiotics, and in many cases complete resistance, is a worldwide phenomenon that is increasing at an alarming rate. Bacteria resistant to the majority of clinically used antibiotics are referred to as "superbugs" and are especially prevalent in communities and hospitals, presenting a great threat. Furthermore, no new antibiotic classes have been discovered over the last 12 years and only a few new antibiotics have been approved as drugs since 1963. "The end of the antibiotic era" emphasizes the importance of continuously developing new antimicrobial agents or investigating alternative approaches for fighting "superbugs" (Hancock, 2007).

Staphylococcus aureus, a "superbug", is a Gram-positive organism that belongs to the Micrococcaceae family. This organism is an opportunistic pathogen associated with a variety of respiratory tract infections, including chronic sinusitis (Brook, 2005), acute otitis media (Grzegorowski and Szydlowski, 2005), healthcare-associated pneumonia (Harmanci *et al.,* 2002) and community-acquired pneumonia (Micek *et al.,* 2007). *S. aureus* is also associated with skin and soft tissue infections (Brook, 2008). Multidrug-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) cause major health care problems, particularly in hospitals and communities. MRSA strains are also resistant to macrolides, lincosamides, flouroquinlones, tetracyclines, aminoglycosides and chloramphenicol (Almer *et al.,* 2002). Even more alarmingly, *S. aureus* strains have been isolated that are resistant to newly developed antimicrobial agents such as quinupristin-dalfopristin, linezolid, daptomycin and tigecycline (Fagon *et al.,* 2000; Peeters and Sarria, 2005; Skiest, 2006).

In South Africa *S. aureus,* and especially multidrug-resistant *S. aureus* strains, are a major health problem. A study conducted in Cape Town investigated the presence of potential bacterial pathogens in 203 HIV-infected children. *S. aureus* was present in 20.4% of the children, of which 77% of the strains were methicillin-resistant (Cotton *et al.,* 2008). *S. aureus* is responsible for serious secondary bacterial infections in HIV-infected individuals as illustrated by another study also conducted in Cape Town. *S. aureus* was found to be the

causative agent in 14% of serious bacterial infections in 141 hospitalized HIV-infected children. All the strains were methicillin-resistant and were predominantly associated with pneumonia (Jaspan *et al.,* 2008).

Lactic acid bacteria (LAB) and the antimicrobial agents they produce have been considered as a possible solution to the problem of controlling multidrug-resistant pathogens (Gillor and Ghazaryan, 2007; Sleator and Hill, 2008). LAB are either rod or coccoid shaped asporogenous cells that grow under micro-aerophylic conditions. Lactic acid is the main end product of the strictly fermentative metabolism (Axelsson, 2004). The ribosomally synthesized antimicrobial peptides they produce are known as bacteriocins and are small cationic proteins with high isoelectric points and amphiphilic characteristics. These proteins generally exhibit antimicrobial activity against strains closely related to the producer strains (Tagg *et al*., 1976).

Lantibiotics falls into one of the bacteriocins' subclasses and are characterized by the presence of lanthionine rings and dehydrated residues. Nisin is probably the most widely known lantibiotic and is the only bacteriocin approved for commercial use. Nisin is usually produced by strains of *Lactococcus lactis* subsp. *lactis.* However, nisin U and U2 are produced by *Streptococcus uberis* (Breukink and de Kruijff, 1999; Wirawan *et al.,* 2006). Gram-positive bacteria, including foodborne pathogens and clinical strains of the genera *Bacillus, Clostridium, Listeria* and *Staphylococcus* are usually inhibited (Breukink and de Kruijff, 1999). Nisin was originally commercialized for food preservation in products such as processed cheese and cheese spreads (Delves-Broughton *et al.,* 1996).

Recently the application of nisin as an antimicrobial in the medical and veterinary fields has been investigated. Nisin has successfully been used in the treatment of mastitis, i.e. intramammary *S. aureus* infections, in cows (Cao *et al.,* 2007; Wu *et al.,* 2007). Mastitis in women has also successfully been treated with nisin (Fernández *et al.,* 2008). Nisin has been incorporated in topical formulations used for the treatment of *S. aureus* infections in atopic dermatitis, but has only been tested *in vitro* (Valenta *et al.,* 1996). Research on microbicides containing nisin has also been conducted (Aranha *et al.,* 2004; Reddy *et al.,* 2004). Nisin was also administered to the nares of cotton rats. The attempted decolonization of *S. aureus* was unsuccessful, probably due to the absorption of nisin in the nasal tract (Kokai-Kun *et al.,*

2003). *In vivo* studies investigating nisin as an alternative to antibiotics are, despite its enormous potential, very limited.

The aim of this study was to characterize nisin F and investigate whether this lantibiotic has an antimicrobial effect *in vivo* against *S. aureus* related respiratory tract and skin infections. This is an initial study on the possible medical applications of nisin F.

References

Almer, L. S., V. D. Shortridge, A. M. Nilius, J. M. Beyer, N. B. Soni, M. H. Bui, G. G. Stone, and R. K. Flamm. 2002. Antimicrobial susceptibility and molecular characterization of community-acquired methicillin-resistant *Staphylococcus aureus*. Diagn. Microbiol. Infect. Dis. **43:**225-232.

Aranha, C., S. Gupta, and K. V. Reddy. 2004. Contraceptive efficacy of antimicrobial peptide nisin: *in vitro* and *in vivo* studies. Contraception **69:**333-338.

Axelsson, L. 2004. Lactic acid bacteria: classification and physiology. *In* S. Salminen, A. von Wright, and A. Ouwehand (ed.), Lactic acid bacteria. Microbiological and functional aspects., Third ed. Marcel Dekker, Inc., New York.

Breukink, E., and B. de Kruijff. 1999. The lantibiotic nisin, a special case or not? Biochim. Biophys. Acta **1462:**223-234.

Brook, I. 2005. Microbiology and antimicrobial management of sinusitis. J. Laryngol. Otol. **119:**251-258.

Brook, I. 2008. Microbiology and management of soft tissue and muscle infections. Int. J. Surg. **6:**328-338.

Cao, L. T., J. Q. Wu, F. Xie, S. H. Hu, and Y. Mo. 2007. Efficacy of nisin in treatment of clinical mastitis in lactating dairy cows. J. Dairy Sci. **90:**3980-3985.

Cotton, M. F., E. Wasserman, J. Smit, A. Whitelaw, and H. J. Zar. 2008. High incidence of antimicrobial resistant organisms including extended spectrum beta-lactamase producing Enterobacteriaceae and methicillin-resistant *Staphylococcus aureus* in nasopharyngeal and blood isolates of HIV-infected children from Cape Town, South Africa. BMC Infect. Dis. **8:**40.

Delves-Broughton, J., P. Blackburn, R. J. Evans, and J. Hugenholtz. 1996. Applications of the bacteriocin, nisin. Antonie van Leeuwenhoek **69:**193-202.

Fagon, J., H. Patrick, D. W. Haas, A. Torres, C. Gibert, W. G. Cheadle, R. E. Falcone, J. D. Anholm, F. Paganin, T. C. Fabian, and F. Lilienthal. 2000. Treatment of Gram-positive nosocomial pneumonia. Prospective randomized comparison of quinupriston/dalfopristin versus vancomycin. Nosocomial Pneunomia Group. Am. J. Respir. Crit. Care Med. **161:**753-762.

Fernández, L., S. Delgado, H. Herrero, A. Maldonado, and J. M. Rodríguez. 2008. The bacteriocin nisin, an effective agent for the treatment of staphylococcal mastitis during lactation. J. Hum. Lact. **24:**311-316.

Gillor, O., and L. Ghazaryan. 2007. Recent advances in bacteriocin application as antimicrobials. Recent Patents Anti-Infect. Drug Disc. **2:**115-122.

Grzegorowski, M., and J. Szydlowski. 2005. Acute otitis media in children. Pol. Merkur. Lekarski **19:**494-496.

Hancock, R. E. W. 2007. The end of an era? Nature Rev. Drug Disc. 6:28.

Harmanci, A., O. Harmanci, and M. Akova. 2002. Hospital-acquired pneumonia: challenges and options for diagnosis and treatment. J. Hosp. Infect. **51:**160-167.

Jaspan, H. B., L. C. Huang, M. F. Cotton, A. Whitelaw, and L. Myer. 2008. Bacterial disease and antimicrobial susceptibility patterns in HIV-infected, hospitalized children: a retrospective cohort study. PLoS ONE. **3:**3260.

Kokai-Kun, J. F., S. M. Walsh, T. Chanturiya, and J. J. Mond. 2003. Lysostaphin cream eradicates *Staphylococcus aureus* nasal colonization in a cotton rat model. Antimicrob. Agents Chemother. **47:**1589-1597.

Micek, S. T., K. E. Kollef, R. M. Reichley, N. Roubinian, and M. H. Kollef. 2007. Health care-associated pneumonia and community-acquired pneumonia: a single center experience. Antimicrob. Agents Chemother. **51:**3568-3573.

Peeters, M. J., and J. C. Sarria. 2005. Clinical characteristics of linezolid-resistant *Staphylococcus aureus* infections. Am. J. Med. Sci. **330:**102-104.

Reddy, K. V., C. Aranha, S. M. Gupta, and R. D. Yedery. 2004. Evaluation of antimicrobial peptide nisin as a safe vaginal contraceptive agent in rabbits: *in vitro* and *in vivo* studies. Reproduction **128:**117-126.

Skiest, D. J. 2006. Treatment failure resulting from resistance of *Staphylococcus aureus* to daptomycin. J. Clin. Microbiol. **44:**655-656.

Sleator, R. D., and C. Hill. 2008. Battle of the bugs. Science **321:**1294-1295.

Tagg, J. R., A. S. Dajani, and L. W. Wannamaker. 1976. Bacteriocins of Gram-positive bacteria. Bacteriol. Rev. **40:**722-756.

Valenta, C., A. Bernkop-Schnürch, and H. P. Rigler. 1996. The antistaphylococcal effect of nisin in a suitable vehicle: a potential therapy for atopic dermatitis in man. J. Pharm. Pharmacol. **48:**988-991.

Wirawan, R. E., N. A. Klesse, R. W. Jack, and J. R. Tagg. 2006. Molecular and genetic characterization of a novel nisin variant produced by *Streptococcus uberis*. Appl. Environ. Microbiol. **72:**1148-1156.

Wu, J., S. Hu, and L. Cao. 2007. Therapeutic effect of nisin Z on subclinical mastitis in lactating cows. Antimicrob. Agents Chemother. **51:**3131-3135.

Chapter 2

Literature review

2.1. *Staphylococcus aureus* and its association with respiratory tract and skin infections

Staphylococcus aureus is a pathogen that belongs to the family Micrococcaceae and cells form characteristics coccoid Gram-positive clusters. Colonies are gold-coloured and strains usually ferment mannitol. *S. aureus* is highly adaptive and grow as a commensal microorganism or as a pathogen in various infections. *S. aureus* is increasingly associated with respiratory tract and skin infections, especially in hospitals and communities. This is due to a range of virulence factors and the increasing prevalence of multi-drug resistant *S. aureus* strains, resulting in treatment failure (Lowy, 1998).

The anterior nares of the human and animal nose (Lowy, 1998; Vautor *et al*., 2005) is the most common site for *S. aureus* infections, followed by the skin, perineum and pharynx (Wertheim *et al.,* 2005). *S. aureus* spreads from the surfaces, were it could survive for extensive periods, to the hands of humans. In hospitals methicillin-resistant *Staphylococcus aureus* (MRSA) outbreaks mainly occur when strains are transmitted from hospital personnel to patients. MRSA strains also infect the nasal cavity through airborne transmission (Farrell *et al*., 1998; Solberg, 2000). The same strains have been isolated from mothers and their children, indicating that transmission also occurs among household members (Peacock *et al.,* 2003). Nasal carriage of *S. aureus* has been associated with an increased risk of subsequent staphylococcal disease (Chapoutot *et al*., 1999; Stanaway *et al*., 2007). The significance of *S. aureus* nasal carriage remains to be debated, since decolonization of *S. aureus* from the nasal tract to prevent staphylococcal disease has only been successful in individuals that have been predisposed to *S. aureus* infections (Kalmeijer *et al*., 2002; Wertheim *et al.,* 2004). One study revealed that the oropharynx of children suffering from cystic fibrosis, and not the nose, is the predominant site of *S. aureus* infection (Ridder-Schaphorn *et al*., 2007).

S. aureus is a causative agent in upper respiratory tract infections such as chronic sinusitis (Brook, 2005), acute otitis media (Grzegorowski and Szydlowski, 2005), tympanostomy tube otorrhea (Francois *et al*., 2001), chronic suppurative otitis media (Verhoeff *et al.,* 2006) and otitis externa (Ramsey, 2002). Upper respiratory tract infections usually occur in stages, starting with a viral infection or allergic inflammation. Complete recovery from infection

occurs in the majority of cases after 10 days (Gwaltney *et al.,* 1981). Facultative aerobic bacteria, i.e. *Streptococcus pneumoniae, Haemophilus influenzae*, *Moraxella catarrhalis,* and *S. aureus* cause secondary acute bacterial infections in cases where viral infections or inflammation were not resolved. Anaerobic bacteria from the oral cavity may predominate over time (Brook *et al.,* 1996; Meyers, 1984).

S. aureus is also associated with lower respiratory tract infections such as cystic fibrosis lung disease, especially in two to three year-old infants, and pneumonia (Baughman *et al.,* 1999; Harmanci *et al.,* 2002; Micek *et al.,* 2007; Saiman, 2004; Stone and Saiman, 2007). Patients with cystic fibrosis have a defective cystic fibrosis transport regulator (CFTR) gene resulting in inconsistent mucus secretion and malfunctioning of the pancreas (Riordan *et al.,* 1989). This in turn leads to colonization of pathogenic bacteria such as *S. aureus* in the lungs, causing inflammation. Chronic inflammation progressively destructs the bronchopulmonary tissue which leads to respiratory insufficiency and death (Kilian and Kisiel, 2006).

S. aureus has been associated with hospital-acquired (nosocomial) and community-acquired pneumonia (Baughman *et al.,* 1999; Harmanci *et al.,* 2002; Micek *et al.,* 2007). Community-acquired pneumonia is often more severe since these strains harbour the Panton-Valentine leukocidin (PVL) gene (Baldwin and Lowe, 2008; Balis *et al.,* 2007). The main route of entry of *S. aureus* into the lungs is by micro-aspiration of oropharyngeal secretions. The oropharyngeal secretions are usually contaminated by bacteria that colonize the upper respiratory tract. After initial colonization with adhesive proteins, discussed later, an infection begins with bronchiolitis that may progress to bronchopneumonia. Bronchopneumonia may be extended to adjacent regions of the lung, resulting in confluent pneumonia, with or without abscess formation (Johanson and Dever, 2003). *S. aureus* has been isolated from these abscesses (Patradoon-Ho and Fitzgerald, 2007).

S. aureus is associated with skin and soft tissue infections (SSTIs) that manifests as cellulitis, boils and abscesses. These infections, if not treated and prolonged, lead to osteomyelitis and even necrotizing fasciitis. SSTIs usually occur in areas of the body that have been previously injured or compromised (Brook, 2008; Miller *et al.,* 2005). Community-acquired methicillin-resistant *S. aureus* (CA-MRSA) is more prevalent in SSTIs than hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA), 75% compared to 37% (Naimi *et al.,* 2003).

One of two things, at least, must occur for an infection to form in a human. Either a highly virulent microorganism must be introduced, or the host defenses have to be impaired. Factors that can impair a host defense system and predispose an individual to a *S. aureus* infection are listed in Table 1. This is followed by a discussion of a range of virulence factors, i.e. a variety of adhesions on the cell surface, production and secretion of tissue damaging toxins, biofilm formation, and resistance against antimicrobial peptides and medicaments (Mack *et al.,* 2004; Peschel, 2002).

TABLE 1. Factors that predispose humans to *S. aureus* infections

Risk factor	Reference
Diabetes mellitus	Lipsky *et al*. (1987)
Haemodialysis procedures	Kirmani *et al.* (1978)
Continuous peritoneal dialysis for end stage liver disease	Nouwen *et al*. (2006)
Cystic fibrosis	Ulrich *et al*. (1998)
Obesity	Herwaldt *et al.* (2004)
Human immunodeficiency virus (HIV) infection	Sissolak *et al.* (2002)
Previous *S. aureus* skin infections	Williams *et al*. (1998)
Intubation and prolonged mechanical ventilation	Pujol *et al.* (1998)
Length of hospitalization or prolonged stay in intensive care unit	Pujol *et al.* (1998)
Previous broad-spectrum antibiotic treatment	Pujol *et al.* (1998)
Atopic dermatitis	Benenson *et al.* (2005)
Intravenous drug usage	González *et al.* (2003)
Surgery	Homer-Vanniasinkam (2007)
Trauma	Brook (2008)
Burns	Blyth (2008)

S. aureus has to adhere to the epithelial cells of the nasal cavity to colonize and cause infection. Strains isolated from nasal carriers harbour genes that encode collagen adhesins (*cna*) and fibronectin-binding proteins (*fnb*A and *fnb*B) (Nashev *et al.,* 2004). Endothelial cells infected with strains containing *fnbA* and *fnbB* release higher levels of interleukin-6, in comparison to cells infected with mutants of *S. aureus* deficient in *fnbA* and *fnbB* (Söderquist *et al.,* 2006). Genotyping of *S. aureus* strains isolated from patients suffering from SSTIs revealed the presence of adhesion genes *map*, *eap* and *fnbB* (Campbell *et al.,* 2008). *S. aureus*

uses teichoic acid, a surface-exposed polymer, to attach to human epithelial cells (Weidenmaier *et al.,* 2004). Sigma B also plays a role in the early infection of *S. aureus* strains by enhancing the cell's ability to attach to fibrinogen- or fibronectin-coated surfaces (Entenza *et al.,* 2005). Another surface-expressed protein, clumping factor B, enhances colonization by adhering to epidermal cytokeratins (O'Brien *et al.,* 2002). *S. aureus,* in addition to these surface-expressed proteins, also secretes proteins that enhance binding to various cells such as Map (Kreikemeyer *et al*., 2002), Eap (Hussain *et al*., 2001a), Emp (Hussain *et al.,* 2001b) or Efb (Bodén and Flock, 1994).

Single chain staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin are important virulence factors that knock out specific subsets of T cells of the host's immune system (Fleischer *et al.,* 1991; Poindexter and Schlievert, 1986). *S. aureus* strains isolated from stable nasal carriers harbour genes coding for enterotoxins SEA, SEB, SEC, SED and SEJ, and TSST-1 (Fueyo *et al*., 2005; Nashev *et al.,* 2004). The strains may also harbour the enterotoxin gene cluster (*egc*) that encode enterotoxins SEG, SEI, SEM, SEN, and SEO (Bania *et al.,* 2006). Clinical strains isolated from SSTIs harbour genes that encode enterotoxins SEA, SEB, SED, SEG, SEJ and SEE (Campbell *et al.,* 2008; Schlievert *et al.,* 2008), toxic shock syndrome toxin (Lina *et al.,* 1997; Schlievert *et al*., 2008) and exfoliative toxins A and B (Lina *et al.,* 1997).

S. aureus strains producing the PVL toxin have been associated with necrotising pneumonia and furunculosis (Lina *et al*., 1999). Necrotising pneumonia is characterized by abscess formation, cavitation, haemorrhage, necrosis and a mortality rate as high as 75% (Gillet *et al*., 2002). Furunculosis is a skin lesion associated with a painful inflammatory reaction and involves the hair follicle (Brook, 2008). PVL-producing *S. aureus* attaches itself to exposed collagen of damaged epithelial. Rapid bacterial multiplication follows with the secretion of cytotoxins, i.e. haemolysins, which causes tissue damage and bacterial spreading (de Bentzmann *et al.,* 2004). When the cells reach a high concentration a quorum-sensing system accessory gene regulator induces the expression of protease and cytotoxic alpha toxins. Leukocidin protects the cells by destroying polymorphs, and is with other necrotising toxins, responsible for necrotising vasculitis with massive areas of infarction and haemorrhage (Seeger *et al.,* 1990).

S. aureus strains are able to form biofilms in the respiratory tract (Amaral *et al*., 2005; Sanderson *et al*., 2006) and on the skin of humans (Smith *et al*., 2008). The first step in biofilm formation is attachment of the cells to biomaterial such as mucus. The mature biofilm is established after the bacteria have formed multiple layers and glycocalyx. The phenotypic characteristics of cells in biofilms are altered with respect to bacterial physiology, metabolism and gene transcription (Donlan and Costerton, 2002; Yarwood *et al*., 2007). Such biofilms complicate therapeutic treatment because of acquired resistance to a variety of antimicrobials and the host's immune system (Mack *et al.,* 2004).

S. aureus is resistant to defensins, cathelicidins and thrombocidins, collectively known as cationic antimicrobial proteins (CAMPs). These compounds may play an important part in the innate immune system, as they form the first line of defense during bacterial colonization and infection (Jin *et al.,* 2004; Medzhitov and Janeway, 2000). CAMPs have cationic properties to target anionic microbial substances and so limit damage to host cells (Fedtke *et al.,* 2004). *MprF* encodes resistance against defensins by modifying the membrane lipids with l-lysine (Peschel *et al.,* 2001). The *dlt* operon also builds resistance against CAMPs by the incorporation of D-alanine into *S. aureus* teichoic acids. Teichoic acids are major components of the *S. aureus* cell wall (Peschel *et al*., 1999). Both these modifications decrease the negative surface charge of the cell walls of bacterial cells and in turn fewer CAMPs bind to *S. aureus* cells. Another important CAMP that *S. aureus* is resistant to is lysozyme. The gene responsible for lysozyme resistance is the peptidoglycan O-acetyltransferase *OatA* (Bera *et al*., 2005). *S. aureus* also produces the exoprotein staphylokinase that forms a complex with alpha-defensins, rendering them ineffective (Jin *et al*., 2004). Braff *et al.* (2007) hypothesized that staphylokinase uses cathelicidins to promote fibrinolysis and enhance bacterial dissemination, hence invasive infection.

The majority of antibiotics available in the pharmaceutical industry today originated from natural templates certain species of microorganisms produced to render them a natural competitive advantage. Antibiotic resistance developed in other microorganisms for survival against these toxic chemicals. Microorganisms become resistant either by mutations or by exchanging genetic resistance determinants with other microorganisms (Hancock, 2005). This is clearly illustrated with *S. aureus* which continuously acquires resistance to newly developed antibiotics.

Methicillin, a semi-synthetic penicillin, was developed to overcome the problem of penicillin-resistant *S. aureus* strains. Methicillin was only efficient for a short period of time with resistant strains detected soon after the antibiotic was introduced (Ayliffe, 1997). The spread of MRSA strains increased rapidly worldwide. As in the case of British bacteraemia patients, 2% of *S. aureus* strains were methicillin-resistant in the early 1990s. This increased to 43% between 1997 and 2002 (Johnson *et al.,* 2005). Methicillin resistance amongst *S. aureus* strains occurs mainly in hospitals and communities and is labeled as hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) and community-acquired methicillin-resistant *S. aureus* (CA-MRSA). HA-MRSA and CA-MRSA differ genetically, in their antibiotic susceptibility profiles and their spectrum of associated infection (Moellering *et al.,* 2007).

Glycopeptides, i.e. vancomycin and teicoplanin, are used as standard treatment for *S. aureus* infections. Vancomycin is the first choice of treatment, especially when dealing with MRSA infections. Alternative antibiotics such as rifampicin, tetracyclines and fusidic acid are also used, depending on the infection site and the susceptibility profile of the microorganism (Casey *et al.,* 2007).

Vancomycin, like other glycopeptides, inhibits the biosynthesis of peptidoglycan in bacterial cell walls (Groves *et al.,* 1994). The occurrence of MRSA strains with reduced susceptibility towards vancomycin is on the rise (Howden, 2005; Ruef, 2004). Vancomycin-sensitive MRSA strains were found to be less susceptible to vancomycin after persistent infection. This reduced susceptibility is associated with a thickening of the cell wall, reduced autolytic activity and a reduction in biofilm-forming ability (Howden *et al.,* 2006). Even more alarming, is the isolation of seven *S. aureus* strains resistant to vancomycin, all from USA (Appelbaum, 2006; Chang *et al.,* 2003). The transfer of plasmids containing the *vanA* gene cluster from vancomycin-resistant enterococci is responsible for resistance in the seven *S. aureus* strains (Weigel *et al.,* 2007).

The decreased susceptibility of *S. aureus* strains to glycopeptides and methicillin has led to the development and registration of the new antistaphylococcal agents; quinupristin-dalfopristin, linezolid, daptomycin and tigecycline. Quinupristin and dalfopristin are two semisynthetic streptogramin derivates that acts synergistically against MSSA and MRSA when combined in a 30:70 (w/w) ratio (Ling *et al.,* 2001). Quinupristin-dalfopristin inhibits protein synthesis by binding onto the bacterial 50S ribosomal subunit. Dalfopristin binds

initially to the ribosome peptidyl site which enhances subsequent binding of quinupristin to a separate but overlapping site (Johnston *et al.*, 2002; Vannuffel and Cocito, 1996). Resistance to quinupristin-dalfopristin is low due to the synergistic action of the two structurally unrelated compounds. Quinupristin resistance is mediated by 23S rRNA target methylation by members of the erythromycin resistance methylase gene (*erm*) class and by linearization of the hexadepsipeptide ring by a specific plasmid-mediated lyase (Klastersky, 2003; Thal and Zervos, 1999). Dalfopristin resistance is mediated by plasmid-mediated acetyltransferases that utilize acetyl-coenzyme A to enzymatically inactivate the compound (Kehoe *et al*., 2003). Recently *S. aureus* strains resistant and less susceptible to quinupristin-dalfopristin have been isolated (Despande *et al.,* 2004; Hsueh *et al.,* 2005).

Linezolid, an oxazolidinone, is bacteriostatic against MSSA and MRSA. Linezolid binds to the 23S ribosomal RNA of the 50S ribosomal subunit, blocking the formation of the ribosomal initiation complex and hence protein synthesis. Cross resistance between linezolid and other protein inhibitors, i.e. chloramphenicol, is infrequent because of linezolid's unique mechanism of action (Diekema and Jones, 2001). Rare cases of acquired resistance have been found in clinical *S. aureus* strains, isolated from patients with indwelling prosthetic devices (Gonzales *et al.,* 2001; Kola *et al.,* 2007). Point mutations in domain V of the 23 rRNA of the 50S ribosomal subunit are responsible for this acquired resistance (Gonzales *et al.,* 2001).

Daptomycin, a cyclic peptide antimicrobial, is bactericidal against MSSA and MRSA. Daptomycin inserts itself into the cell membrane of sensitive cells causing the membrane to depolarize and to release its potassium ions. This in turn results in the inhibition of DNA, RNA, and protein synthesis (Silverman *et al.,* 2003). *S. aureus* strains resistant to daptomycin are extremely rare (Hayden *et al*., 2005; Mangili *et al.,* 2005; Marty *et al.,* 2006).

Tigecycline is a glycylcycline antibiotic with a fused tetracycline, minocylnine structure and is modified by the addition of a 9-*t*-butylglycylamido side chain (Doan *et al.,* 2006). Tigecyclines binds to the 30S ribosomal subunit, blocking the addition of amino acids to polypeptide chains and hence inhibit protein synthesis. Tigecycline is a broad-spectrum antibiotic with bacteriostatic activity against MSSA and MRSA (Fritsche *et al.,* 2005).

The isolation of *S. aureus* strains resistant to the newly registered antistaphylococcal agents, although rare, is still a concern. This drives the need for the continuous exploration of new

antimicrobial agents, of which the following drugs are in the advanced stages of clinical testing. Dalbavacin, oritavacin and telavancin are second-generation glycopeptides with a prolonged half-live, active against MRSA strains (Jábes *et al.,* 2004; Stryjewski *et al.,* 2005; Van Bambeke, 2006). Ramoplanin, a lipoglycodepsipeptide, is another newly developed antibiotic with activity against multi-drug resistant *S. aureus* strains. Ramoplanin interferes with the biosynthesis and transglycosylation of lipid II in the cell wall of bacterial cells (Hu *et al.,* 2003; Somner and Reynolds, 1990). Other examples are ceftobiprole, ceftaroline, iclaprim, CS-023/RO-4908463, and adjuvant therapies, i.e. monoclonal antibody tefibazumab (Pan *et al*., 2008).

2.2. Bacteriocins with emphasis on nisin

Bacteriocins are gene-encoded peptides that exhibit antimicrobial properties against other bacterial species that are usually closely related to the producer strain. Lactic acid bacteria (LAB) are well known for their production of bacteriocins. These Gram-positive, nonsporing, nonrespiring cocci or rods follow a strictly fermentative metabolism with lactic acid as end product (Axelsson, 2004). Bacteriocins produced by LAB are small cationic proteins with high iso-electric points and amphiphilic characteristics (Tagg *et al*., 1976). Klaenhammer (1993) originally classified bacteriocins into four distinct classes, based on their biochemical and genetic characteristics, structures and mechanisms of action. Recently, Cotter *et al.* (2005a) reclassified bacteriocins into two groups i.e. Class I, lantibiotics and Class II, nonlantibiotics (Table 2).

TABLE 2. Classification of bacteriocins (Cotter *et al.,* 2005a)

Class	Characteristics	Example	Reference
Class I	Small (< 5 kDa), heat stable posttranslationally modified peptides containing lanthionine and methyllanthionine amino acids		Chatterjee *et al.* (2005); Jack and Sahl (1995)
Class Ia	Elongated, flexible positively charged peptides. Forms pores in cytoplasmic membranes of sensitive cells	nisin	Breukink and de Kruijff (1999)

Class	Characteristics	Example	Reference
Class Ib	Globular, rigid peptides with a negative or no net charge. Interfere with essential enzymatic reactions of sensitive cells	mersacidin	Bierbaum *et al.* (1995)
Class II	Small (< 10 kDa) heat stable nonlanthionine containing peptides		
Class IIa	Pediocin-like peptides with conserved YGNGVXCXXXXXCXV region in N-terminal domain	pediocin AcH/PA-1	Eijsink *et al.* (1998)
Class IIb	Two-component bacteriocins requiring two peptides for activity	lactococcin G	Nissen-Meyer *et al.* (1992)

Nisin is a small, ribosomally synthesized, antimicrobial peptide produced by certain *Lactococcus lactis* strains. The exception is nisin U that is produced by *Streptococcus uberis* (Breukink and de Kruijff, 1999; Wirawan *et al.,* 2006). *Lactococcus lactis* and *Streptococcus uberis* are LAB that previously formed part of the genus *Streptococcus*. This genus has since been divided into three separate taxons, namely *Enterococcus*, *Lactococcus* and *Streptococcus sensu stricto* (Schleifer, 1987).

Mattick and Hirsch (1947) discovered the first lantibiotic, nisin A, but the structure was only published a few years later by Gross and Morell (1971). Since then, many lantibiotics have been discovered, among them five other natural variants of nisin Z, Q, F, U and U2. Nisin A, Z, Q and F have similar activities, but differ only in a few amino acids. Nisin A and nisin Z differ only in a single amino acid and nisin A from nisin Q in six amino acids (Buchman *et al.,* 1988; Mulders *et al*., 1991; Zendo *et al.,* 2003). Nisin A differs from nisin F in two amino acids and nisin F differs from nisin Q in four amino acids (de Kwaadsteniet *et al.,* 2008). Nisin U is only 78% identical to nisin A and Z and 82% identical to nisin Q (Wirawan *et al.,* 2006).

Nisin has antimicrobial activity against various Gram-positive bacteria, including *Bacillus, Clostridium, Listeria* and *Staphylococcus* spp. (Breukink and de Kruijff, 1999). This peptide is part of the lantibiotic family because of the lanthionine rings and dehydrated residues in its structure (Buchman *et al.,* 1988). Lantibiotics are divided into two groups as mentioned earlier, the elongated type A group and the globular type B group with nisin falling into the

first group (Table 2). Nisin is a cationic peptide with amphipathic properties. The dehydrated residues are formed posttranslational when serine and threonine are dehydrated to respectively dehydroalanine (Dha) and dehydrobutyrine (Dhb). The lanthionine rings are then formed when five of the dehydrated residues are coupled to upstream cysteines. These cysteines are then converted to alanine and the S used to form the thioether bonds (Fig. 3). These thioether bonds form a N-terminally and a C-terminally ring system that is separated by a hinge region (residues 20 - 22). Nisin is now positioned in a screw-like structure that is responsible for its amphipathic characteristics (Fig. 3).

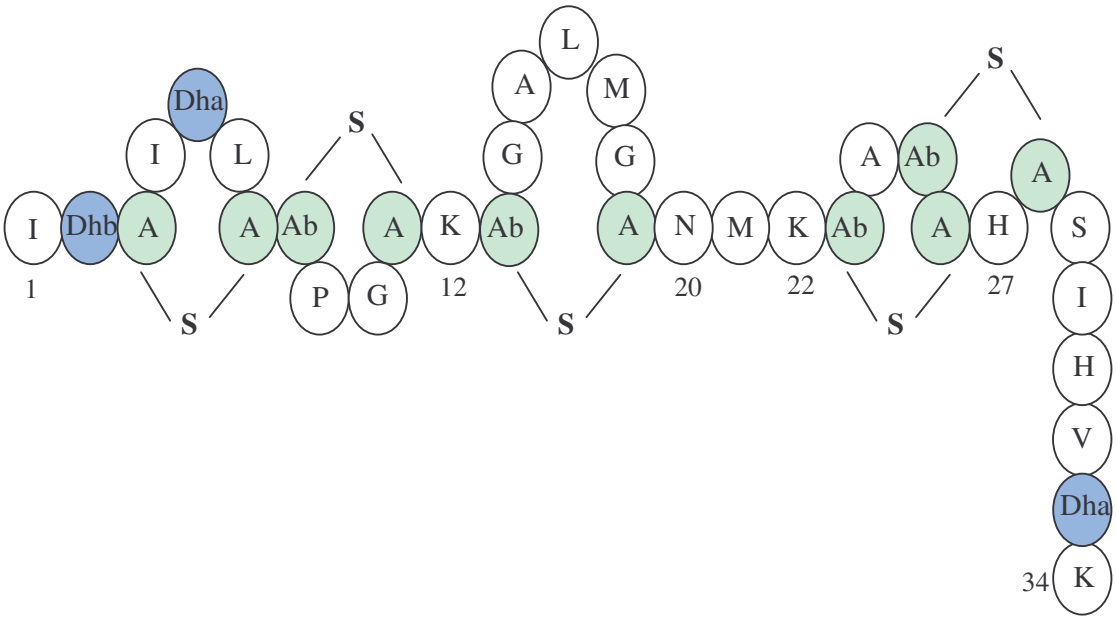

Fig. 1. The primary structure of nisin A. The lanthionine rings (A-**S**-A, lanthionine; Abu-**S**-Ala, methyllanthionine) and the dehydrated residues (Dhb, dehydroalanine; Dha, dehydrobutyrine) are respectively coloured in green and blue (Breukink and de Kruijff, 1999).

The nisin A operon has been studied in detail. The nisin gene cluster consists out of 11 genes, i.e. *nisA, nisB, nisT, nisC, nisI, nisP, nisR, nisK, nisF, nisE* and *nisG* (Fig. 4). This gene cluster is situated on a conjugative plasmid, together with *sacA* encoding sucrose-6-phosphate hydrolase (Rauch and de Vos, 1992). Transposons that vary slightly form strain-to-strain flank the operon and the *sacA* gene. These transposons are transferred to enterococci through conjugation (Broadbent *et al.,* 1995). The structural gene *nisA* encodes the prepeptide (Buchman *et al.,* 1988). *NisB* and *nisC* are involved in maturation of the lantibiotic and *nisT* in transport across the cell membrane (Engelke *et al*., 1992). *NisI* encodes an immunity

protein and *nisP* a putative serine protease involved in processing (Engelke *et al.,* 1994). *NisR* and *nisK* encode a regulatory protein and a histidine kinase (Engelke *et al.,* 1994). *NisF*, *nisE* and *nisG* encode ATP-binding cassette (ABC) transporters that play a role in immunity together with *nisI* (Siegers and Entian, 1995; Stein *et al.,* 2003).

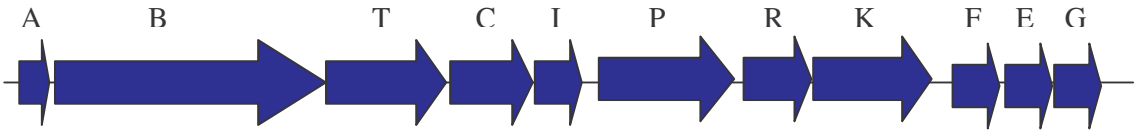

Fig. 2. The nisin gene cluster consisting out of eleven genes (blue arrows) namely *nisA, nisB, nisT, nisC, nisI, nisP, nisR, nisK, nisF, nisE* and *nisG* (Engelke *et al.,* 1994; Stein *et al.,* 2003).

The mode of action is the formation of voltage-dependent pores in the plasmamembrane of target cells, which allows the efflux of potassium ions, ATP and amino acids. The membrane-bound cell wall precursor lipid II plays an important role in the pore formation since it acts as nisin's docking molecule (van Heusden *et al.,* 2002). Nisin bounded to lipid II further increases its effectiveness as an antimicrobial by inhibiting bacterial cell wall synthesis (Wiedemann *et al.,* 2001).

Klaenhammer (1993) defined three categories of nisin resistance for Gram-positive bacteria. Firstly, nisin-producing strains are protected from its own bacteriocin with the immunity genes *nisI* and *nisFEG* (Engelke *et al.,* 1994; Siegers and Entian, 1995). Secondly, nisinases (nisin-inactivating enzymes) have been isolated from non-nisin-producing Gram-positive strains (Jarvis and Farr, 1971). Lastly, is the acquirement of nisin resistance. A variety of different mechanisms have been observed among Gram-positive sensitive strains that acquired nisin resistance. Nisin resistance in *Listeria* strains coincided with a thickening in the cell wall (Maisnier-Patin and Richard, 1996) and changes in the fatty acid composition of the cell membrane (Mazzotta and Montville, 1997). Lipoteichoic acids in the cell walls of *S. aureus* and *Streptococcus bovis* are associated with nisin-resistance (Mantovani and Russell, 2001; Peschel *et al.,* 1999). A putative binding protein is also associated with nisin-resistance in *L. monocytogenes* strains (Gravesen *et al.,* 2001). The complexity by which sensitive Gram-positive bacteria acquire nisin resistance was studied by Kramer *et al.* (2006). Transcriptional analysis revealed that 92 genes in *L. lactis* IL1403 are either directly or

indirectly involved in acquired nisin resistance. The following four mechanisms are involved: (i) preventing nisin from reaching the cytoplasmic membrane and lipid II molecule; (ii) stimulating the binding of nisin to the cell wall by increasing the pH of the extracellular medium which promotes degradation and immobilization; (iii) preventing nisin insertion into the membrane and (iv) ATP-transporters may possibly be involved in the extrusion of nisin from the cytoplasmic membrane. Bacteria may use more than one mechanism simultaneously to protect themselves against nisin (Kramer *et al.,* 2006).

Nisin has generally regarded as safe (GRAS) status and inhibit food borne pathogens (Delves-Broughton *et al.,* 1996; Rilla *et al*., 2004). Numerous toxicology studies proved that nisin has a low toxicity (Fowler, 1973; Lloyd and Drake, 1975). The American Food and Drug Administration (Federal Register, 1988) and a joint commission of the Food and Agriculture Organization and World Health Organization (WHO, 1969) have approved nisin A for certain applications in food. Nisin inhibits spore outgrowth (Chan *et al.,* 1996) and is mainly used to protect processed cheese and cheese spreads from contamination (Delves-Broughton *et al.,* 1996).

2.3. The application of bacteriocins in the medical field

Bacteriocins and bacteriocin-producing LAB are being intensively studied since their antimicrobial activity against pathogens render them as possible alternatives to antibiotics. *Clostridium difficile* is a common cause of hospital-acquired diarrhoea associated with a high mortality (Pépin *et al*., 2005). Lacticin 3147 (Rea *et al.,* 2007) and nisin (Bartoloni *et al.,* 2007) have been shown to be antimicrobial against clinical *C. difficile* strains. Lantibiotics such as lacticins (Kim *et al.,* 2003) and nisin (Morency *et al.,* 2001) also inhibit *Helicobacter pylori,* a gastric pathogen contributing to gastroduodenal ulcers and gastric adenocarcinoma (Kandulski *et al.,* 2008). The spent culture supernatants of two *Lactobacillus acidophilus* strains, LB and La1, were shown to protect mice against *Helicobacter felis* (Coconnier *et al.,* 1998) and humans against *H. pylori* infection (Gotteland *et al.,* 2008; Gotteland and Cruchet, 2003). Enterocin CR35, produced by *E. faecium* CRL35, is antimicrobial against herpes simplex virus (HSV) (Wachsmans *et al.,* 1999) and inhibits the replication of the virus *in vitro* (Wachsmans *et al.,* 2003). Nisin has been shown to be antimicrobial towards *S. aureus* (Wang *et al.,* 2005). Even MRSA strains have shown a loss in viability after treatment with nisin alone (Brumfitt *et al*., 2002; Severina *et al.,* 1998) and nisin combined with ramoplanin

(Brumfitt *et al.,* 2002). Other bacteriocins have also been shown to have antimicrobial activity against *S. aureus* strains *in vitro* (Table 3).

TABLE 3. Bacteriocins produced by LAB active against *S. aureus* strains *in vitro*

Bacteriocin	Reference
Lactococcus sp.	
Nisin	Brumfitt *et al*. (2002)
Lacticin 3147	Galvin *et al.* (1999)
Diacetin B	Ali *et al.* (1995)
Lactobacillus sp.	
Plantaricin ST31	Todorov *et al.* (1999)
Plantaricin TF711	Hernández *et al.* (2005)
Plantaricin 149	Müller *et al.* (2007)
Bacteriocin 217	Lozo *et al.* (2004)
Bacteriocin LS1	Busarcevic *et al.* (2008)
Salivacin 140	Arihara *et al.* (1996)
Reutericyclin	Gänzle *et al.* (2000)
Pediococcus sp.	
Pediocin A	Piva and Headon (1994)
Pediocin AcH	Bhunia *et al.* (1988)
Leuconostoc sp.	
Mesentericin ST99	Todorov and Dicks (2004)
Enterococcus sp.	
Enterocin EJ97	Gálvez *et al*. (1998)
Enterocin CCM 4231	Lauková *et al.* (2001)
Enterocin AS-48	Ananou *et al.* (2004)
Enterocin E-760	Line *et al.* (2008)
Enterocin P	Cintas *et al.* (1997)
Carnobacterium sp.	
Carnocin H	Blom *et al.* (2001)

Despite the enormous therapeutic potential of bacteriocins, the numbers of *in vivo* studies in murine models are limited. Kruszewska *et al*. (2004) investigated the effect of mersacidin on *S. aureus* in a mouse rhinitis model. Mersacidin, a lantibiotic produced by *Bacillus* sp. HIL Y-

85, 54728, inhibits the growth of *S. aureus in vitro* (Bierbaum *et al*., 1995). The immune system of the mice was compromised by hydrocortisone administration before the nasal tract was infected with MRSA. *S. aureus* was not detected in the nasal scrapings of mersacidin-treated mice, indicating that mersacidin administered intranasally was successful in eradicating the infection. Furthermore, only mice not treated with mersacidin had detectable levels of interleukin-1β in their blood. The absence of this immune reaction in treated mice supports the therapeutical potential of mersacidin (Kruszewska *et al.,* 2004). Nisin applied in the nasal tract of cotton rats was not able to eradicate or decrease *S. aureus* carriage. The same concentration nisin exhibited antistaphylococcal activity *in vitro,* suggesting that nisin is either inactivated or absorbed in the nasal tract (Kokai-Kun *et al.,* 2003).

Nisin has been incorporated in topical formulations used in the treatment of *S. aureus* infections in atopic dermatitis (Valenta *et al.,* 1996). The use of nisin as a safe vaginal contraceptive has also been investigated (Aranha *et al.,* 2004; Reddy *et al.,* 2004). Lacticin 3147 and subtilosin A have also been marked as potential spermicidal candidates (Silkin *et al.,* 2008). Mastitis in women and cows have been successfully treated with nisin (Cao *et al.,* 2007; Fernández *et al.,* 2008; Wu *et al.,* 2007) and lacticin 3147 (Crispie *et al.,* 2005; Ryan *et al.,* 1999). Nisin preparations commercially available to treat bovine mastitis include Consept (Applied Microbiology, Inc., New York) and Wipe-Out (ImmuCell, Portland, Oregon) (Cotter *et al.,* 2005b). The combination of nisin and RNAIII-inhibiting peptide was shown to inhibit *S. epidermis* infection on implanted grafts in Wistar rats (Ghiselli *et al.,* 2004).

Bacteriocins such as nisin and lacticin 3147 are degraded in the intestinal tract and can therefore not be taken orally (Bernbom *et al.,* 2006; Gardiner *et al.,* 2007). This hurdle can be overcome by the development of "designer probiotics". Hill and Sleator proposed the cloning and expression of bacteriocin genes in a probiotic carrier such as *Lactobacillus salivarius*, enhancing its clinical efficacy (Hill and Sleator, 2008). The alternative is oral administration of bacteriocin-producing strains, although they are not of intestinal origin or known probiotics. Lacticin 3147- producing *L. lactis* and nisin-producing *L. lactis* strains were shown to survive intestinal transit in animal models (Bernbom *et al.,* 2008; Dobson *et al.,* 2008). Nisin has also been incorporated in tablets using pectin/HPMC polymer mixtures and was shown to remain active for six hours *in vitro*, approximately the colon transit time (Ugurlu *et al.,* 2007). The importance of bacteriocin production in probiotic strains is illustrated by a groundbreaking study by Corr *et al.* (2007). *Lactobacillus salivarius* UCC118

producing the bacteriocin Abp118 protected mice against *Listeria monocytogenes* infection. A mutant non-bacteriocin producing *L. salivarius* UCC118 strain failed to protect mice. Also, *L. salivarius* UC118 was ineffective against a mutant *L. monocytogenes* strain that was able to produce the Abp118 immunity protein.

2.4. Molecular imaging

Molecular imaging monitors gene expression *in vivo* at cellular and molecular levels, using imaging detectors that target either endogenous or exogenous genes. Optical imaging, radionuclide-based imaging methods (i.e. positron emission tomography and single photon emission computerised tomography) and magnetic resonance imaging (MRI) are all non-invasive imaging techniques used to study host-pathogen interactions.

Newly developed technology enables light produced by biochemical reactions and biological processes in small animals to be detected and quantified. Experimental lay-outs using optical imaging *in vivo* are approached by either using: endogenous fluorochromes; reporter genes that generate light internally from specific sources (either bioluminescence of fluorescent proteins); or injected contrast agents that incorporate visible light fluorophores, near-infrared fluorophores or activated fluorophores (Contag and Ross, 2002). Table 4 lists the advantages and disadvantages of using bioluminescence, fluorescent proteins and targeted fluorescent probes in optical imaging.

TABLE 4: Comparing the three optical methods used for studying host-pathogen interactions

	Advantages	Disadvantages	References
Biolumine-scence	Low signal to noise ratio Detects infection recovery Closely imitates natural bacterial strains Suitable for longitudinal studies	Limited tissue penetration of visible luminescence Availability of oxygen and ATP Bacteria must be encoded with genetic reporter Signal can vary	Doyle *et al.* (2004)

	Advantages	Disadvantages	References
Fluorescent proteins	No substrate needed Closely imitates natural bacterial strains Suitable for longitudinal studies	Limited tissue penetration of visible emission Autofluorescence Bacteria must be encoded with genetic reporter	Shaner *et al.* (2005)
Targeted fluorescent probes	Bright near-infrared probes developed Multimodel probes under development Enzymes can serve as activators	Probe preparation Probe may affect bacterial function Toxicity and stability of probes Not suited for longitudinal studies	Leevy *et al.* (2006)

Positron emission tomography (PET) entails the labeling of a molecule with positron-emitting radionuclides and then administrating these labeled molecules by intravenous injection or inhalation. Radionuclides decay by emitting positrons and their tissue concentration is determined with an imaging device, a PET scanner (Piwnica-Worms *et al.,* 2004). PET imaging have for example been applied in studies investigating inflammatory responses to infectious organisms (Jones *et al*., 1997; Schuster *et al*., 2003). Bacteria or cells can also be directly monitored in living animals if they have been modified to express, for example, herpes simplex virus type-1 thymidine kinase (HSV1-TK) that is detected through radiolabeled ATP analogs (Bettegowda *et al*., 2005).

Magnetic resonance imaging (MRI) entails that protons within an animal host will absorb energy when laying in a magnetic field subjected to a radiofrequency pulse that is applied at the correct (resonance) frequency. A detectable signal is then generated when the radiofrequency pulse is turned off and the protons emit that energy back into the environment. The majority of MRI experiments involve the measurement of protons in water (Piwnica-Worms *et al.,* 2004). MRI can be used to study pathological tissues in small animals such as oedema and inflammation in lungs (Beckman *et al.,* 2001), airway calibre after chronic infection with pathogens, and functional assessment of ventilation and perfusion (Piwnica-Worms *et al.,* 2004). Table 5 lists the advantages and disadvantages of the three molecular techniques.

TABLE 5: Different molecular imaging techniques for studying host-pathogen interactions

	Advantages	Disadvantages	References
Optical imaging	Low in cost Highly versatile Enables multichannel imaging Simple to execute Short image acquisition times Simultaneous imaging of more than one animal	Transmission of light through animals wavelength dependent Tissue attenuation Lacks depth information Low resolution	Piwnica-Worms *et al.* (2004)
Positron emission tomography (PET)	Tomographic imaging capabilities	Expensive Radionuclides are hazardous Low sensitivity Low resolution	Doyle *et al.* (2004); Contag and Ross (2002)
Magnetic resonance imaging	High spatial resolution Three dimensional imaging	Expensive Difficult to execute	Enninga *et al.* (2007)

Although optical imaging has its disadvantages, it is the best molecular imaging technique to use when studying the course of a bacterial infection. PET uses dangerous radionuclides to label bacteria and MRI detects the pathological tissues and not the bacteria.

2.5. Bioluminescent imaging studies (BLI)

Bioluminescence imaging (BLI) is a newly developed methodology that uses the light emitted from living organisms as a tool for molecular imaging in small laboratory animals. BLI makes the real time, noninvasive *in vivo* monitoring of infectious diseases possible. *In vivo* BLI works on the following principle. Cells that express light-producing enzymes, luciferases, are placed within the host animal. In the presence of ATP and oxygen this enzyme emits detectable photons (Doyle *et al.,* 2004; Shah and Weissleder, 2005). BLI systems can capture and analyze both bioluminescence and fluorescence using a specialized charge coupled device (CCD) camera. After capturing the photons the CCD camera converts them into electrons. The CCD camera generates an image by encoding the electrons into electrical charge patterns. Background noise is minimized by super-cooling the CCD camera to -90°C and mounting the

camera in a light-tight box (Sadikot and Blackwell, 2005). Bioluminescence can be detected from genetically engineered microorganisms, cell lines or reporter genes.

There are numerous advantages when using BLI over conventional methods in investigating infectious diseases in small animals. The number of animals needed to obtain statistically meaningful results is drastically reduced when compared to conventional technologies. This is because spatial and temporal data can be collected from the same animal over multiple time points without the need of euthanization. The animal models are more accurate since the data are collected from intact, living animals (Hutchens and Luker, 2007). Problems associated with conventional technologies, such as plate contamination or antibiotics carryover effect, that can influence the experimental outcome are also eliminated (Kadurugamuwa *et al*., 2003b).

Three luciferase enzymes have to date been characterized and applied for *in vivo* BLI studies in living animals, namely luciferase from fireflies (coleoptara) (de Wet *et al.,* 1985), jellyfish and sea pansies (cnidaria) (Hart *et al.,* 1979; Lorenz *et al.,* 1991), and bacteria (Frackman *et al.,* 1990). This review will focus on bacterial luciferases and their applications in studying Gram-positive bacterial infections. Five essential genes are responsible for the ability of bioluminescent bacteria to synthesize light. These genes are organized in an operon such as *luxCDABE*. Additional *lux* genes have been identified from bioluminescent bacteria but only *luxCDABE* is responsible for the biosynthesis of light. *LuxAB* encodes a heterodimeric luciferase that catalyses the oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long-chain fatty aldehyde. This results in blue-green light that is emitted with a peak at 490 nm. *LuxCDE* encodes a fatty acid reductase complex that synthesizes the long-chain fatty aldehydes (Meighen, 1993).

Photorhabdus luminescence is ideally situated for BLI studies in mammalian animal models since the luciferase it produces has a high thermal stability being stable at even 45°C (Meighen, 1993; Szittner and Meighen, 1990). *P. luminescence* belongs to the terrestrial genera *Photorhabdus* and is like all the other luciferase-producing bacteria Gram-negative (Meighen, 1994). The other luciferase-producing bacteria are from marine habitats and belong to the genera *Vibrio, Photobacterium,* and *Shewanella* (Ulitzur, 1997). The *luxCDABE* has been successfully cloned into Gram-negative bacteria such as *Salmonella* and *Escherichia coli* (Contag *et al.,* 1995; Xi *et al*., 1991). Ribosomes in Gram-positive bacteria cannot bind

and translate to mRNA from the *P. luminescens luxCDABE* operon. This makes the translation of the *P. luminescens luxCDABE* operon into Gram-positive bacteria such as *S. aureus* problematic. The partial *lux* operon (*luxAB*) was initially transformed into *S. aureus* with some degree of success (Corbisier *et al.,* 1993). The fact that additional exogenous substrate and detergent are required and that the luciferase is unstable above 30°C, makes this approach not optimal for the use in animal models (Hill *et al.,* 1993).

Francis *et al.* (2000) modified the *luxCDABE* operon so that it could be expressed by Gram-positive bacteria and was renamed *luxABCDE*. This was accomplished by inserting a Gram-positive ribosome binding site (RBS) upstream of each gene and preceding the operon with an appropriate promoter sequence. Several highly bioluminescent strains of *S. aureus* and other Gram-positive bacteria were generated after successful transformation with the plasmid. The technical disadvantages are that plasmid loss will occur in long term studies due to the absence of antibiotics and that plasmid artifacts, such as copy number and supercoiling, which will make monitoring unreliable will occur (Francis *et al.*, 2000). The same research group overcame the problem by constructing a novel Gram-positive *lux* transposon cassette, Tn4001 *luxABCDE* Km^R, which allows the integration of this operon into the chromosome of Gram-positive bacteria. The *luxABCDE* and kanamycin genes were linked to a single promoterless operon (Fig. 5). This design will result in bioluminescence and kanamycin resistance occurring only in a bacterial cell if the operon has transposed downstream of a promoter on the genome (Francis *et al.*, 2001).

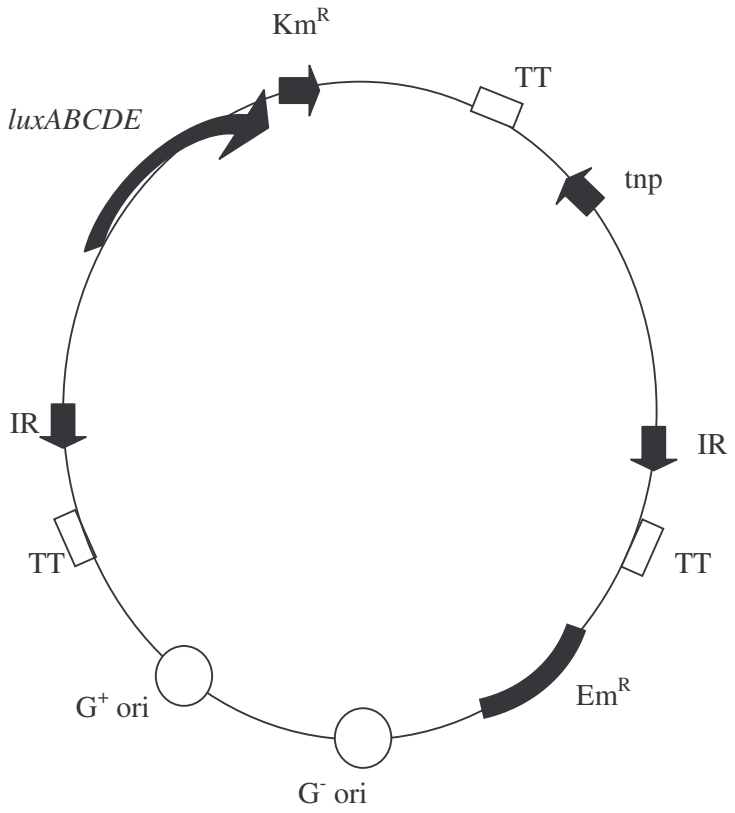

Fig. 3. Plasmid pAUL-A Tn4001 *luxABCDE* Km^R. IR, inverted repeat; EmR, erythromycin resistance gene; tnp, transposase gene; TT, transcription terminator (Francis *et al.,* 2001).

Bioluminescence flux measurements of bioluminescent-engineered bacteria correlate well with viable cell numbers (Francis *et al.,* 2001; Francis *et al.,* 2000; Hamblin *et al*., 2003; Jawhara and Mordon, 2004; Kadurugamuwa *et al*., 2003a; Mortin *et al.,* 2007; Rocchetta *et al.,* 2001). Certain studies have shown that once microorganisms reach stationary phase, viable bioluminescence and viable cell numbers do not correlate anymore (Kadurugamuwa *et al.,* 2003b; Kuklin *et al.,* 2003). Discrepancies were also observed between viable cell numbers and bioluminescence during a *Pseudomonas aeruginosa* biofilm study (Marques *et al*., 2005). In both these cases this can be ascribed to the fact that bioluminescence is an indicator of the metabolic state of a cell since luciferases are energy-requiring oxygenase (Kadurugamuwa *et al.,* 2003b; Marques *et al.,* 2005). Bioluminescence can be detected for days and can even reappear after initial diminishing. This is clearly illustrated by a study performed by Kadurugamuwa *et al.* (2003b). Treating biofilm infections on catheters with different antibiotics were investigated using bioluminescent *S. aureus*. Although a 90% reduction was observed in the rifampin-treated group the intensity of bioluminescence

increased after final treatment. This makes bioluminescent imaging studies (BLI) ideally situated to monitor not only the course of an antimicrobial treatment but also the relapse of the treatment (Fig. 4).

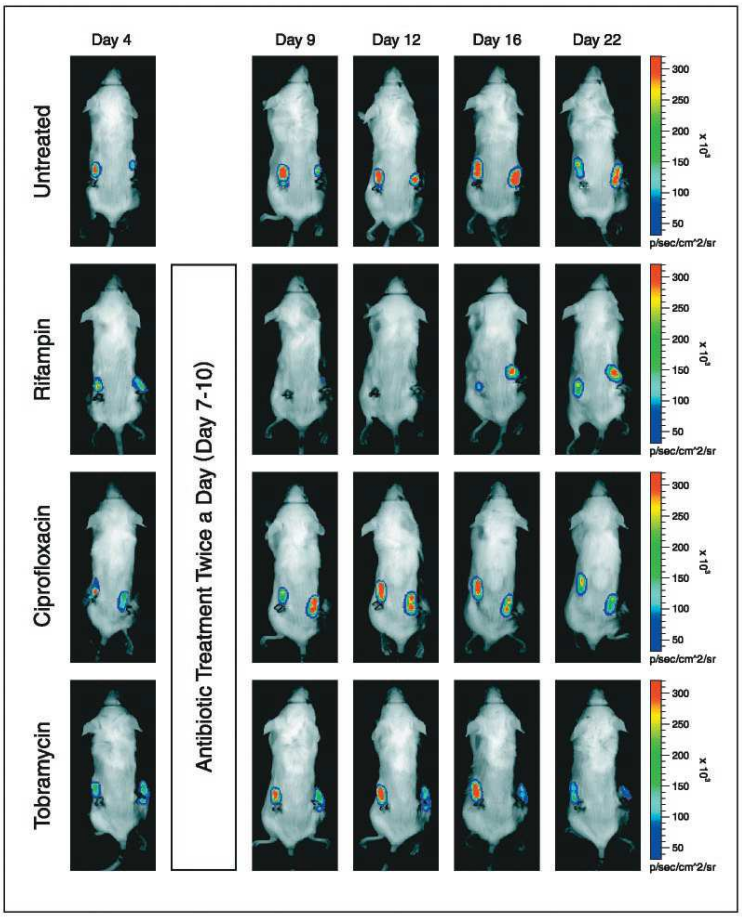

Fig. 4. Bioluminescent *S. aureus* Xen 29 formed biofilms on implanted catheters. The effect of antibiotics on the infections was monitored in real-time by measuring the bioluminscence (photons/sec/cm^2, number of photons per second per square centimeter of catheter) (Kadurugamuwa *et al.,* 2003b).

Bioluminescent reporters have a lower signal to noise ratio compared to fluorescent proteins. This is because the wavelength used to excite fluorescent proteins causes the mammalian tissue to autofluorescence. Mice do not bioluminescence and create a very low signal to noise ratio. There are limiting factors to consider when planning and performing BLI studies. The detection of bioluminescent bacteria is dependent on the wavelength transmission of light through animal tissues. With each centimeter of tissues depth, an approximately 10-fold loss of photon intensity is observed. Another factor to consider, especially in longitudinal studies,

is the availability of oxygen and ATP luciferase needs to oxidize reduced flavin mononucleotide ($FMNH_2$), as mentioned earlier (Sadikot and Blackwell, 2005). Table 6 lists the *in vivo* studies that used BLI to investigate infectious diseases caused by Gram-positive bacteria.

TABLE 6. *In vivo* studies that investigated Gram-positive bacterial infections using BLI

Pathogen	Aim of study	Reference
S. aureus	Investigating the effectiveness of antibiotics in treating infections in the thigh muscles of mice	Francis *et al.* (2000)
S. aureus	Monitoring the effectiveness of antibiotics on biofilms on catheters planted in mice	Kadurugamuwa *et al.* (2003a,b, 2004) ; Yu *et al*. (2005)
S. aureus	Monitoring staphylococcal foreign-body and deep-thigh-wound infections in mice	Kuklin *et al.* (2003)
S. aureus	Investigating the effectiveness of antibiotics in a rat endocarditis model	Xiong *et al.* (2005)
S. aureus	Monitoring implant-associated osteomyelitis infections	Li *et al.* (2008)
S. aureus	Monitoring the spread of a staphylococcal infection on implanted biomaterial	Engelsman *et al.* (2008)
S. aureus	Investigating the antimicrobial activity of daptomycin against *S. aureus* peritonitis infections	Mortin *et al*. (2007)
S. pneumoniae	Investigating antibiotic treatments of infections in the lungs of mice	Francis *et al*. (2001)
S. pneumoniae	Monitoring the spread of the pathogen throughout the central nervous system during acute stages of bacterial meningitis.	Kadurugamuwa *et al.* (2005b,c)
S. pneumoniae	Monitoring the pathogenesis of pneumococcal meningitis simultaneous with the host response	Kadurugamuwa *et al.* (2005a)
S. pneumoniae	Investigating whether virulence determinants play a role the transition of the pathogen between body sites	Orihuela *et al*. (2004)

Pathogen	Aim of study	Reference
L. monocytogenes	Investigating the presence of the pathogen in the human gall bladder where replication occurs	Hardy *et al*. (2004)
L. monocytogenes	Investigating the protective effect of bacteriocin-producing *L. salivarius* UCC118 against the pathogenic infection	Corr *et al*. (2007)

Bioluminescent and fluorescent imaging studies have successfully been applied in other pharmaceutical fields. BLI can monitor tumor growth and metastasis noninvasively. Tumors are also detected earlier with BLI than traditional methods such as ultrasounds and clinical manifestation (Dickson *et al*., 2007; Jurczok *et al*., 2008). The response in murine models to different cancer therapies can also be evaluated using BLI, i.e. an effective treatment will result in a decrease in tumor size which will be detected by a decrease in bioluminescence (Bartlett *et al.,* 2007; Dickson *et al*., 2007; Jurczok *et al*., 2008).

The expression of key genes involved the fields of neurology (Luo *et al.,* 2006; Zhao *et al.,* 2006), cardiovascular (Davidson *et al*., 2005; Prasad *et al*., 2007), immunology (Tolar *et al.,* 2007), inflammation (Flanagan *et al*., 2007) and autoimmune diseases (Tarner *et al.,* 2003) can be monitored using BLI. BLI also allows for the quantitative measurement of endogenous (Quesada *et al.,* 2008) or transplanted (Folwer *et al*., 2005; Roth *et al*., 2006) pancreatic beta cells when studying diabetes.

2.6. Animal models

In vitro models investigate the activity and safety of antimicrobial peptides in static test systems while studies in humans can only assess morphological changes. For obvious reasons clinical trials in humans must abide to strict rules and regulations of ethical committees. Murine models are therefore the essential link between *in vitro* models and clinical trials for evaluating the safety and efficiency of antimicrobial peptides. Murine models provide a complete picture of the response the administration of antimicrobial peptides will have on the immune system, natural microflora and organs (histopathology) of mammals. Another advantage of murine models is that different variables can be manipulated that is not feasible in humans (Bakker-Woudenberg, 2003; Kips *et al*., 2003).

Scientists have used rats and mice as animal models since the eighteenth-century. The laboratory rat was the best functionally described mammalian model system. Rats were used in studies investigating cardiovascular diseases, metabolic disorders, neurological disorders, neurobiological disorders, organ transplantation, autoimmune diseases, cancer susceptibility and renal diseases. Rats have been used in studying diseases in the respiratory tract such as emphysema (Kuraki *et al.,* 2002), influenza (Huang *et al.,* 2004), pulmonary fibrosis (Spond *et al.,* 2003) and pneumonia (Wan *et al*., 2006). Wistar rats were inbreeded from albino rats as early as 1909 by Helen Dean King at the Wistar Institute of Anatomy and Biology in Philadelphia (Krinke, 2000).

The Human Genome Initiative selected the mouse as the first mammal to have its genome sequenced. Even though the physical appearances of humans and mice differ drastically, their genes are approximately 99% identical. The genes in humans and mice also function biologically in the same way. All this and the fact that genome of mice can be easily manipulated makes mice the ideal model organism for studying inherited human disorders (Hedrich, 2004). Mice are ideally situated for BLI studies because of their small size. As mentioned earlier with every centimeter of tissues depth, a 10-fold loss of photon intensity is observed (Sadikot and Blackwell, 2005). Mice have been used in studies investigating infections in the respiratory tract such as cystic fibrosis (Desigaux *et al.,* 2005), lung cancer (Gagnadoux *et al*., 2005), influenza (Ogata and Shibata, 2008) and pneumonia (Scarff and Goldberg, 2008).

Studying *S. aureus* infections in the respiratory tract of mice and rats each has their own unique drawbacks. Rats are less susceptible to *S. aureus* strains of human origin. Mice, on the other hand, are not the best model to use when studying unilateral pneumonia. Unilateral pneumonia has successfully been established in rats (Bakker-Woudenberg *et al*., 2002).

S. aureus- related skin infection have been studied in mice (Abe *et al.,* 1993; Stearne *et al.,* 2002) and rat models (Girard *et al.,* 1993; Pattanayak and Sunita, 2008). Rats eliminate *S. aureus* at a faster rate from wound infections than mice and hamsters. Rats are also more resistant to localized wound infection (Donnelly and Stark, 1985). The skins of rats are found more resistant to staphylococcal exfoliative toxin, associated with staphylococcal scalded skin syndrome, than the skins of mice and humans (Machida, 1995).

Animal models have limitations even though they serve as a basis for clinical research in humans. The virulence and growth of infectious microorganism and the immunological response of the host differs from animals to humans. Furthermore, higher dosage of an antimicrobial agent is needed in rodents to acquire the same efficiency in humans. The reason for this is that rodents eliminate antibiotics at a faster rate. Animal models have also shown to produce slightly varied results as each model have their own set of advantages and disadvantages. Therefore, proper analysis and interpretation of data in animal models for clinical use is optimised when more than one animal model is used and their data compared (Bakker-Woudenberg, 2003).

2.7. References

Abe, Y., H. Akiyama, and J. Arata. 1993. Furuncle-like lesions in mouse experimental skin infections with *Staphylococcus aureus.* J. Dermatol. **20:**198-202.

Ali, D., C. Lacroix, D. Thuault, C. M. Bourgeois, and R. E. Simard. 1995. Characterization of diacetin B, a bacteriocin from *Lactococcus lactis* subsp. *lactis* bv. *diacetylactis* UL720. Can. J. Microbiol. **41:**832-841.

Amaral, M. M., L. R. Coelho, R. P. Flores, R. R. Souza, M. C. Silva-Carvalho, L. A. Teixeira, B. T. Ferreira-Carvalho, and A. M. Figueiredo. 2005. The predominant variant of the Brazilian epidemic clonal complex of methicillin-resistant *Staphylocccus aureus* has an enhanced ability to produce biofilm and to adhere to and invade airway epithelial cells. J. Infect. Dis. **192:**801-810.

Ananou, S., E. Valdivia, M. Martínez Bueno, A. Gálvez, and M. Maqueda. 2004. Effect of combined physico-chemical preservatives on enterocin AS-48 activity against the enterotoxigenic *Staphylococcus aureus* CECT 976 strain. J. Appl. Microbiol. **97:**48-56.

Appelbaum, P. C. 2006. The emergence of vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. Clin. Microbiol. Infect. **12:**16-23.

Aranha, C., S. Gupta, and K. V. Reddy. 2004. Contraceptive efficacy of antimicrobial peptide nisin: *in vitro* and *in vivo* studies. Contraception **69:**333-338.

Arihara, K., S. Ogihara, T. Mukai, M. Itoh, and Y. Kondo. 1996. Salivacin 140, a novel bacteriocin from *Lactobacillus salivarius* subsp. *salicinius* T140 active against pathogenic bacteria. Lett. Appl. Microbiol. **22:**420-424.

Axelsson, L. 2004. Lactic acid bacteria: classification and physiology. *In* S. Salminen, A. von Wright, and A. Ouwehand (ed.), Lactic acid bacteria. Microbiological and functional aspects., Third ed. Marcel Dekker, Inc., New York.

Ayliffe, G. A. 1997. The progressive intercontinental spread of methicillin-resistant *Staphylococcus aureus*. Clin. Infect. Dis. **24:**S74-S79.

Bakker-Woudenberg, I. A. 2003. Experimental models of pulmonary infection. J. Microbiol. Methods **54:**295-313.

Bakker-Woudenberg, I. A., M. T. ten Kate, L. Guo, P. Working, and J. W. Mouton. 2002. Ciprofloxacin in polyethylene glycol-coated liposomes: efficacy in rat models of acute or chronic *Pseudomonas aeruginosa* infection. Antimicrob. Agents Chemother. **46:**2575-2581.

Baldwin, L. N., and A. D. Lowe. 2008. Panton-Valentine leukocidin associated with community acquired methicillin resistant *Staphylococcus aureus*: a case report and review of interim guidelines. Anaesthesia **63:**764-766.

Balis, E., C. Diacaki, P. Tselioti, D. Perimeni, S. Vourli, A. Vatopoulos, H. Giamarellou, and A. Prekates. 2007. Community-acquired pneumonia and bacteremia due to methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine-leukocidin gene in Greece: two case reports and literature review. J. Chemother. **19:**703-708.

Bania, J., A. Dabrowska, K. Korzekwa, A. Zarczynska, J. Bystron, J. Chrzanowska, and J. Molenda. 2006. The profiles of enterotoxin genes in *Staphylococcus aureus* from nasal carriers. Lett. Appl. Microbiol. **42:**315-320.

Bartlett, D. W., H. Su, I. J. Hildebrandt, W. A. Weber, and M. E. Davis. 2007. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles meaured by multimodality *in vivo* imaging. Proc. Natl. Acad. Sci. U.S.A. **104:**15549-15554.

Bartoloni, A., A. Mantella, B. P. Goldstein, R. Dei, M. Benedetti, S. Sbaragli, and F. Paradisi. 2004. *In-vitro* activity of nisin against clinical isolates of *Clostridium difficile*. J. Chemother. **16:**119-121.

Baughman, R. P., V. Tapson, and A. McIvor. 1999. The diagnosis and treatment challenges in nosocomial pneumonia. Diagn. Microbiol. Infect. Dis. **33:**131-139.

Beckmann, N., B. Tigani, D. Ekatodramis, R. Borer, L. Mazzoni, and J. R. Fozard. 2001. Pulmonary edema induced by allergen challenge in the rat: noninvasive assessment by magnetic resonance imaging. Magn. Reson. Med. **45:**88-95.

Benenson, S., O. Zimhony, D. Dahan, M. Solomon, D. Raveh, Y. Schlesinger, and A. M. Yinnon. 2005. Atopic dermatitis – a risk factor for invasive *Staphylococcus aureus* infections: two cases and review. Am. J. Med. **118:**1048-1051.

Bera, A., S. Herbert, A. Jakob, W. Vollmer, and F. Götz. 2005. Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan O-acetyltransferase OatA is the major determinant for lysozyme resistance of *Staphylococcus aureus*. Mol. Microbiol. **55:**778-787.

Bernbom, N., T. R. Licht, C. H. Brogren, B. Jelle, A. H. Johansen, I. Badiola, F. K. Vogensen, and B. Nørrung. 2006. Effects of *Lactococcus lactis* on composition of intestinal microbiota: role of nisin. Appl. Environ. Microbiol. **72:**239-244.

Bettegowda, C., C. A. Foss, I. Cheong, Y. Wang, L. Diaz, N. Agrawal, J. Fox, J. Dick, L. H. Dang, S. Zhou, K. W. Kinzler, B. Vogelstein, and M. G. Pomper. 2005. Imaging bacterial infections with radiolabeled 1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-5-iodouracil. Proc. Natl. Acad. Sci. U.S.A. **102:**1145-1150.

Bhunia, A. K., M. C. Johnson, and B. Ray. 1988. Purification, characterization and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. J. Appl. Bacteriol. **65:**261-268.

Bierbaum, G., H. Brötz, K. P. Koller, and H. G. Sahl. 1995. Cloning, sequencing and production of the lantibiotic mersacidin. FEMS Microbiol. Lett. **127:**121-126.

Blom, H., T. Katla, H. Nissen, and H. Holo. 2001. Characterization, production, and purification of carnocin H, a bacteriocin produced by Carnobacterium 377. Curr. Microbiol. **43:**227-231.

Blyth, M., C. Estela, and A. E. R. Young. 2008. Severe staphylococcal scalded skin syndrome in children. Burns **34:**98-103.

Bodén, M. K., and J. I. Flock. 1994. Cloning and characterization of a gene for a 19 kDa fibrinogen-binding protein from *Staphyloccus aureus*. Mol. Microbiol. **12:**599-606.

Braff, M. H., A. L. Jones, S. J. Skerrett, and C. E. Rubens. 2007. *Staphylococcus aureus* exploits cathelicidin antimicrobial peptides produced during early pneumonia to promote staphylokinase-dependent fibrinolysis. J. Infect. Dis. **195:**1365-1372.

Breukink, E., and B. de Kruijff. 1999. The lantibiotic nisin, a special case or not? Biochim. Biophys. Acta **1462:**223-234.

Broadbent, J. R., W. E. Sandine, and J. K. Kondo. 1995. Characteristics of Tn5307 exchange and intergeneric transfer of genes associated with nisin production. Appl. Microbiol. Biotechnol. **44:**139-146.

Brook, I. 2005. Microbiology and antimicrobial management of sinusitis. J. Laryngol. Otol. **119:**251-258.

Brook, I. 2008. Microbiology and management of soft tissue and muscle infections. Int. J. Surg. **6:**328-338.

Brook, I., E. H. Frazier, and P. A. Foote. 1996. Microbiology of the transition from acute to chronic maxillary sinusitis. J. Med. Microbiol. **45:**372-375.

Brumfitt, W., M. R. Salton, and J. M. Hamilton-Miller. 2002. Nisin, alone and combined with peptidoglycan-modulating antibiotics: activity against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. J. Antimicrob. Chemother. **50:**731-734.

Buchman, G. W., S. Banerjee, and J. N. Hansen. 1988. Structure, expression, and evolution of a gene encoding the precursor of nisin, a small protein antibiotic. J. Biol. Chem. **263:**16260-16266.

Busarcevic, M., M. Kojic, M. Dalgalarrondo, J. M. Chobert, T. Haertlé, and L. Topisirovic. 2008. Purification of bacteriocin LS1 produced by human oral isolate *Lactobacillus salivarius* BGH01. Oral Microbiol. Immunol. **23:**254-258.

Campbell, S. J., H. S. Deshmukh, C. L. Nelson, I. G. Bae, M. E. Stryjewski, J. J. Federspiel, G. T. Tonthat, T. H. Rude, S. L. Barriere, R. Corey, and V. G. Jr. Folwer. 2008. Genotypic characteristics of *Staphylococcus aureus* isolates from a multinational trial of complicated skin and skin structure infections. J. Clin. Microbiol. **46:**678-684.

Cao, L. T., J. Q. Wu, F. Xie, S. H. Hu, and Y. Mo. 2007. Efficacy of nisin in treatment of clinical mastitis in lactating dairy cows. J. Dairy Sci. **90:**3980-3985.

Casey, A. L., P. A. Lambert, and T. S. Elliot. 2007. Staphylococci. Int. J. Antimicrob. Agents **3:**S23-S32.

Chan, W. C., H. M. Dodd, N. Horn, K. Maclean, L. Y. Lian, B. W. Bycorft, M. J. Gasson, G. C. Roberts. 1996. Structure-activity relationships in the peptide antibiotic nisin: role of dehydroalanine 5. Appl. Environ. Microbiol. **62:**2966-2969.

Chang, S., D. M. Sievert, J. C. Hageman, M. L. Boulton, F. C. Tenover, F. P. Downes, S. Shah, J. T. Rudrik, G. R. Pupp, W. J. Brown, D. Cardo, S. K. Fridkin, Vancomycin-resistant *Staphylococcus aureus* Investigative Team. 2003. Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. N. Engl. J. Med. **348:**1342-1347.

Chapoutot, C., G. P. Pageaux, P. F. Perrigault, Z. Joomaye, P. Perney, H. Jean-Pierre, O. Jonquet, P. Blanc, and D. Larrey. 1999. *Staphylococcus aureus* nasal carriage in 104 cirrhotic and control patients. A prospective study. J. Hepatol. **30:**249-253.

Chatterjee, C., M. Paul, L. Xie, and W. A. van der Donk. 2005. Biosynthesis and mode of action of lantibiotics. Chem. Rev. **105:**633-684.

Cintas, L. M., P. Casaus, L. S. Håvarstein, P. E. Hernández, and I. F. Nes. 1997. Biochemical and genetic characterization of enterocin P, a novel *sec*-dependent bacteriocin from *Enterococcus faecium* P13 with a broad antimicrobial spectrum. Appl. Environ. Microbiol. **63:**4321-4330.

Coconnier, M. H., V. Lievin, E. Hemery, and A. L. Servin. 1998. Antagonistic activity against *Helicobacter* infection *in vitro* and *in vivo* by the human *Lactobacillus acidophilus* strain LB. Appl. Environ. Microbiol. **64:**4573-4580.

Contag, C. H., P. R. Contag, J. I. Mullins, S. D. Spilman, D. K. Stevenson, and D. A. Benaron. 1995. Photonic detection of bacterial pathogens in living hosts. Mol. Microbiol. **18:**593-603.

Contag, C. H., and B. D. Ross. 2002. It's not just about anatomy: *in vivo* bioluminescence imaging as an eyepiece into biology. J. Magn. Reson. Imaging **16:**378-387.

Corbisier, P., G. Ji, G. Nuyts, M. Mergeay, and S. Silver. 1993. *LuxAB* gene fusions with the arsenic and cadmium resistance operons of *Staphylococcus aureus* plasmid pl258. FEMS Microbiol. Lett. **110:**231-238.

Corr, S. C., Y. Li, C. U. Riedel, P. W. O'Toole, C. Hill, and C. G. Gahan. 2007. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. Proc. Natl. Acad. Sci. U.S.A. **104:**7617-7621.

Cotter, P. D., C. Hill, and R. P. Ross. 2005a. Bacteriocins: developing innate immunity for food. Nat. Rev. Microbiol. **3:**777-788.

Cotter, P. D., C. Hill, and R. P. Ross. 2005b. Bacterial lantibiotics: strategies to improve therapeutic potential. Curr. Protein Pept. Sci. **6:**61-75.

Crispie, F., D. Twomey, J. Flynn, C. Hill, P. Ross, and W. Meaney. 2005. The lantibiotic lacticin 3147 produced in a milk-based medium improves the efficacy of a bismuth-based teat seal in cattle deliberately infected with *Staphylococcus aureus*. J. Dairy Res. **72:**159-167.

Davidson, A. J., B. London, G. D. Block, and M. Menaker. 2005. Cardiovascular tissues contain independent circadian clocks. Clin. Exp. Hypertens. **27:**307-311.

de Bentzmann, S., A. Tristan, J. Etienne, N. Brousse, F. Vandenesch, and G. Lina. 2004. *Staphylococcus aureus* isolates associated with necrotizing pneumonia bind to basement membrane type I and IV collagens and laminin. J. Infect Dis. **190:**1506-1515.

de Kwaadsteniet, M., Ten Doeschate, K. and L. M. Dicks. 2008. Characterization of the structural gene encoding nisin F, a new lantibiotic produced by a *Lactococcus lactis* subsp. *lactis* isolate from freshwater fish (*Clarius gariepinus*). Appl. Environ. Microbiol. **74:**547-549.

de Wet, J. R., K. V. Wood, D. R. Helinski, and M. DeLuca. 1985. Cloning of firefly luciferase cDNA and the expression of active luciferase in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **82:**7870-7873.

Delves-Broughton, J., P. Blackburn, R. J. Evans, and J. Hugenholtz. 1996. Applications of the bacteriocin, nisin. Antonie Van Leeuwenhoek **69:**193-202.

Deshpande, L. M., T. R. Fritsche, and R. N. Jones. 2004. Molecular epidemiology of selected multidrug-resistant bacteria: a global report from the SENTRY Antimicrobial Surveillance Program. Diagn. Microbiol. Infect. Dis. **49:**231-236.

Desigaux, L., C. Gourden, M. Bello-Roufaï, P. Richard, N. Oudrhiri, P. Lehn, D. Escande, H. Pollard, and B. Pitard. 2005. Nonionic amphiphilic block copolymers promote gene transfer to the lung. Hum. Gene Ther. **16:**821-829.

Dickson, P. V., B. Hamner, C. Y. Ng, M. M. Hall, J. Zhou, P. W. Hargrove, M. B. McCarville, and A. M. Davidoff. 2007. *In vivo* bioluminescence imaging for early detection and monitoring of disease progression in a murine model of neuroblastoma. J. Pediatr. Surg. **42:**1172-1179.

Diekema, D. J., and R. N. Jones. 2001. Oxazolidinone antibiotics. Lancet **358:**1975-1982.

Doan, T. L., H. B. Fung, D. Mehta, and P. F. Riska. 2006. Tigecycline: a glycylcycline antimicrobial agent. Clin. Ther. **28:**1079-1106.

Dobson, E., F. Crispie, C. Rea, E. Gardiner, G. Casey, G. Lawlor, C. Hill, and R. P. Ross. 2008. The fate of the lacticin 3147-producing *Lactococcus lactis* in the mammalian gastrointestinal tract. The 9th Symposium on lactic acid bacteria: health, evolution and systems biology. Abstract book D074.

Donlan, R. M., and J. W. Costerton. 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev. **15:**167-193.

Donnelly, T. M., and D. M. Stark. 1985. Susceptibility of laboratory rats, hamsters, and mice to wound infections with *Staphylococcus aureus*. Am. J. Vet. Res. **46:**2634-2638.

Doyle, T. C., S. M. Burns, and C. H. Contag. 2004. *In vivo* bioluminescence imaging for integrated studies of infection. Cell. Microbiol. **6:**303-317.

Eijsink, V. G., M. Skeie, P. H. Middelhoven, M. B. Brurberg, and I. F. Nes. 1998. Comparative studies of class IIa bacteriocins of lactic acid bacteria. Appl. Environ. Microbiol. **64:**3275-3281.

Engelke, G., Z. Gutowski-Eckel, M. Hammelmann, and K. D. Entian. 1992. Biosynthesis of the lantibiotic nisin: genomic organization and membrane localization of the nisB protein. Appl. Environ. Microbiol. **58:**3730-3743.

Engelke, G., Z. Gutowski-Eckel, P. Kiesau, K. Siegers, M. Hammelmann, and K. D. Entian. 1994. Regulation of nisin biosynthesis and immunity in *Lactococcus lactis* 6F3. Appl. Environ. Microbiol. **60:**814-825.

Engelsman, A. F., H. C. van der Mei, K. P. Francis, H. J. Busscher, R. J. Ploeg, and G. M. van Dam. 2008. Real time noninvasive monitoring of contaminating bacteria in a soft tissue implant infection model. J. Biomed. Mater. Res. B. Appl. Biomater. **In press.**

Enninga, J., P. Sansonetti, and R. Tournebize. 2007. Roundtrip explorations of bacterial infection: from single cells to the entire host and back. Trends Microbiol. **15:**483-490.

Entenza, J. M., P. Moreillon, M. M. Senn, J. Kormanec, P. M. Dunman, B. Berger-Bächi, S. Projan, and M. Bischoff. 2005. Role of sigmaB in the expression of *Staphylococcus aureus* cell wall adhesions ClfA and FnbA and contribution to infectivity in a rat model of experimental endocarditis. Infect. Immun. **73:**990-998.

Farrell, A. M., D. C. Shanson, J. S. Ross, N. M. Roberts, C. Fry, J. J. Cream, R. C. Staughton, and C. B. Bunker. 1998. An outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) in a dermatology day-care unit. Clin. Exp. Dermatol. **23:**249-253.

Federal Register. 1988. Nisin preparation: affirmation of GRAS status as a direct human food ingredient. Fed. Reg. **53:**11247-11251.

Fedtke, I., F. Götz, and A. Peschel. 2004. Bacterial evasion of innate host defenses - the *Staphylococcus aureus* lesson. Int. J. Med. Microbiol. **294:**189-194.

Fernández, L., S. Delgado, H. Herrero, A. Maldonado, and J. M. Rodríguez. 2008. The bacteriocin nisin, an effective agent for the treatment of staphylococcal mastitis during lactation. J. Hum. Lact. **24:**311-316.

Flanagan, J. M., J. Truksa, H. Peng, P. Lee, and E. Beutler. 2007. *In vivo* imaging of hepcidin promoter stimulation by iron and inflammation. Blood Cells Mol. Dis. **38:**253-257.

Fleischer, B., R. Gerardy-Schahn, B. Metzroth, S. Carrel, D. Gerlach, and W. Köhler. 1991. An evolutionary conserved mechanism of T cell activation by microbial toxins. Evidence for different affinities of T cell receptor-toxin interaction. J. Immunol. **146:**11-17.

Fowler, G. G. 1973. Toxicology of nisin. Food Cosmet. Toxicol. **11:**351-352.

Fowler, M., J. Virostko, Z. Chen, G. Poffenberger, A. Radhika, M. Brissova, M. Shiota, W. E. Nicholson, Y. Shi, B. Hirshberg, D. M. Harlan, E. D. Jansen, and A. C. Powers. 2005. Assessment of pancreatic islet mass after islet transplantation using *in vivo* bioluminescence imaging. Transplantation **15:**768-776.

Frackman, S., M. Anhalt, and K. H. Nealson. 1990. Cloning, organization, and expression of the bioluminescence genes of *Xenorhabdus luminescens*. J. Bacteriol. **172:**5767-5773.

Francis, K. P., D. Joh, C. Bellinger-Kawahara, M. J. Hawkinson, T. F. Purchio, and P. R. Contag. 2000. Monitoring bioluminescent *Staphylococcus aureus* infections in living mice using a novel *luxABCDE* construct. Infect. Immun. **68:**3594-3600.

Francis, K. P., J. Yu, C. Bellinger-Kawahara, D. Joh, M. J. Hawkinson, G. Xiao, T. F. Purchio, M. G. Caparon, M. Lipsitch, and P. R. Contag. 2001. Visualizing pneumococcal infections in the lungs of live mice using bioluminescent *Streptococcus pneumoniae* transformed with a novel Gram-positive *lux* transposon. Infect. Immun. **69:**3350-3358.

Francois, M., P. Mariani-Kurkdjian, C. Doit, P. Benzekri, E. Bingen, and J. G. Macé. 2001. Tympanostomy tube otorrhea: microbiological epidemiology, pathogen susceptibility to local antibiotics and bactericidal power of the rifamycin-olfloxacin combination. Ann. Otolaryngol. Chir. Cervicofac. **118:**278-282.

Fritsche, T. R., H. S. Sader, M. G. Stilwell, M. J. Dowzicky, and R. N. Jones. 2005. Potency and spectrum of tigecycline tested against an international collection of bacterial pathogens associated with skin and soft tissue infections (2000-2004). Diagn. Microbiol. Infect. Dis. **52:**195-201.

Fueyo, J. M., M. C. Mendoza, and M. C. Martin. 2005. Enterotoxins and toxic shock syndrome toxin in *Staphylococcus aureus* recovered from human nasal carries and manually handled foods: epidemiological and genetic findings. Microbes Infect. **7:**187-194.

Gagnadoux, F., A. L. Pape, E. Lemarié, S. Lerondel, I. Valo, V. Leblond, J. L. Racineux, and T. Urban. 2005. Aerosol delivery of chemotherapy in an orthotopic model of lung cancer. Eur. Respir. J. **26:**657-661.

Gálvez, A., E. Valdivia, H. Abriouel, E. Camafeita, E. Mendez, M. Martínez-Bueno, and M. Maqueda. 1998. Isolation and characterization of enterocin EJ97, a bacteriocin produced by *Enterococcus faecalis* EJ97. Arch. Microbiol. **171:**59-65.

Galvin, M., C. Hill, and R. P. Ross. 1999. Lacticin 3147 displays activity in buffer against Gram-positive bacterial pathogens which appear insensitive in standard plate assays. Lett. Appl. Microbiol. **28:**355-358.

Gänzle, M. G., A. Höltzel, J. Walter, G. Jung, and W. P. Hammes. 2000. Characterization of reutericyclin produced by *Lactobacillus reuteri* LTH2584. Appl. Environ. Microbiol. **66:**4325-4333.

Gardiner, G. E., M. C. Rea, B. O'Riordan, P. O'Connor, S. M. Morgan, P. G. Lawlor, P. B. Lynch, M. Cronin, R. P. Ross, and C. Hill. 2007. Fate of two-component lantibiotic 3147 in the gastrointestinal tract. Appl. Environ. Microbiol. **73:**7103-7109.

Ghiselli, R., A. Giacometti, O. Cirioni, G. Dell'Acqua, F. Mocchegiani, F. Orlando, G. D'Amato, M. Rocchi, G. Scalise, and V. Saba. 2004. RNAIII-inhibiting peptide and/or nisin inhibit experimental vascular graft infection with methicillin-susceptible and methicillin-resistant *Staphylococcus epidermis*. Eur. J. Vasc. Endovasc. Surg. **27:**603-607.

Gillet, Y., B. Issartel, P. Vanhems, J. C. Fournet, G. Lina, M. Bes, F. Vandenesch, Y. Piémont, N. Brousse, D. Floret, and J. Etienne. 2002. Association between *Staphylocccus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet **359:**753-759.

Girard, D., J. M. Bergeron, W. B. Milisen, and J. A. Retsema. 1993. Comparison of azithromycin, roxithromycin, and cephalexin penetration kinetics in early and mature abscesses. J. Antimicrob. Chemother. **31:**17-28.

González, C., M. Rubio, J. Romero-Vivas, M. González, and J. J. Picazo. 2003. *Staphylococcus aureus* bacteremic pneumonia: differences between community and nosocomial acquisition. Int. J. Infect. Dis. **7:**102-108.

Gonzales, R. D., P. C. Schreckenberger, M. B. Graham, S. Kelkar, K. DenBesten, and J. P. Quinn. 2001. Infections due to vancomycin-resistant *Enterococcus faecium* resistant to linezolid. Lancet **357:**1179.

Gotteland, M., and S. Cruchet. 2003. Suppressive effect of frequent ingestion of *Lactobacillus johnsonii* La1 on *Helibacter pylori* colonization in asymptomatic volunteers. J. Antimicrob. Chemother. **51:**1317-1319.

Gotteland, M., M. Andrews, M. Toledo, L. Muñoz, P. Caceres, A. Anziani, E. Wittig, H. Speisky, and G. Salazar. 2008. Modulation of *Helicobacter pylori* colonization with cranberry juice and *Lactobacillus johnsonii* La1 in children. Nutrition **24:**421-426.

Gravesen, A., K. Sørensen, F. M. Aarestrup, and S. Knøchel. 2001. Spontaneous nisin-resistant *Listeria monocytogenes* mutants with increased expression of a putative penicillin-binding protein and their sensitivity to various antibiotics. Microb. Drug Resist. **7:**127-135.

Gross, E., and J. L. Morell. 1971. The structure of nisin. J. Am. Chem. Soc. **93:**4634-4635.

Groves, P., M. S. Searle, J. P. Mackay, and D. H. Williams. 1994. The structure of an asymmetric dimer relevant to the mode of action of the glycopeptides antibiotics. Structure **8:**747-754.

Grzegorowski, M., and J. Szydlowski. 2005. Acute otitis media in children. Pol. Merkur. Lekarski **19:**494-496.

Gwaltney, J. M. Jr., A. Jr. Sydnor, and M. A. Sande. 1981. Etiology and antimicrobial treatment of acute sinusitis. Ann. Otol. Rhinol. Laryngol. Suppl. **90:**68-71.

Hamblin, M. R., T. Zahra, C. H. Contag, A. T. McManus, and T. Hasan. 2003. Optical monitoring and treatment of potentially lethal wound infections *in vivo*. J. Infect. Dis. **187:**1717-1725.

Hancock, R. E. 2005. Mechanisms of action of newer antibiotics for Gram-positive pathogens. Lancet Infect. Dis. **5:**209-218.

Hardy, J., K. P. Francis, M. DeBoer, P. Chu, K. Gibbs, and C. H. Contag. 2004. Extracellular replication of *Listeria monocytogenes* in the murine gall bladder. Science **303:**851-853.

Harmanci, A., O. Harmanci, and M. Akova. 2002. Hospital-acquired pneumonia: challenges and options for diagnosis and treatment. J. Hosp. Infect. **51:**160-167.

Hart, R. C., J. C. Matthews, K. Hori, and M. J. Cormier. 1979. *Renilla reniformis* bioluminescence: luciferase-catalyzed production of nonradiating excited states from luciferin analogues and elucidation of the excited state species involved in energy transfer to Renilla green fluorescent protein. Biochemistry **18:**2204-2210.

Hayden, M. K., K. Rezai, R. A. Hayes, K. Lolans, J. P. Quinn, and R. A. Weinstein. 2005. Development of daptomycin resistance *in vivo* in methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **43:**5285-5287.

Hedrich, H. 2004. The laboratory mouse. Elsevier Academic Press, London, UK.

Hernández, D., E. Cardell, and V. Zárate. 2005. Antimicrobial activity of lactic acid bacteria isolated from Tenerife cheese: initial characterization of plantaricin TF711, a bacteriocin-like substance produced by *Lactobacillus plantarum* TF711. J. Appl. Microbiol. **99:**77-84.

Herwaldt, L. A., J. J. Cullen, P. French, J. Hu, M. A. Pfaller, R. P. Wenzel, and T. M. Perl. 2004. Preoperative risk factors for nasal carriage of *Staphylococcus aureus*. Infect. Control Hosp. Epidemiol. **25:**481-484.

Hill, P. J., C. E. Rees, M. K. Winson, and G. S. Stewart. 1993. The application of *lux* genes. Biotechnol. Appl. Biochem. **17:**3-14.

Homer-Vanniasinkam, S. 2007. Surgical site and vascular infections: treatment and prophylaxis. Int. J. Infect. Dis. **11:**S17-S22.

Howden, B. P. 2005. Recognition and management of infections caused by vancomycin-intermediate *Staphylococcus aureus* (VISA) and heterogenous VISA (hVISA). Intern. Med. J. **35:**136-140.

Howden, B. P., P. D. Johnson, P. B. Ward, T. P. Stinear, and J. K. Davies. 2006. Isolates with low-level vancomycin resistance associated with persistent methicillin-resistant *Staphylocccus aureus* bacteremia. Antimicrob. Agents Chemother. **50:**39-47.

Hsueh, P.-R., W.-H. Chen, and K.-T. Luh. 2005. Nosocomial infections due to methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci at a university hospital in Taiwan from 1991 to 2003: resistance trends, antibiotic usage and *in vitro* activities of newer antimicrobial agents. Int. J. Antimicrob. Agents **26:**43-49.

Hu, Y., J. S. Helm, L. Chen, X. Y. Ye, and S. Walker. 2003. Ramoplanin inhibits bacterial transglycosylases by binding as a dimer to lipid II. J. Am. Chem. Soc. **23:**8736-8737.

Huang, J., R. J. Garmise, T. M. Crowder, K. Mar, C. R. Hwang, A. J. Hickey, J. A. Mikszta, and V. J. Sullivan. 2004. A novel dry powder influenza vaccine and intranasal delivery technology: induction of systematic and mucosal immune responses in rats. Vaccine **21:**794-801.

Hussain, M., K. Becker, C. von Eiff, G. Peters, and M. Herrmann. 2001a. Analogs of Eap protein are conserved and prevalent in clinical *Staphylococcus aureus* isolates. Clin. Diagn. Lab. Immunol. **8:**1271-1276.

Hussain, M., K. Becker, C. von Eiff, J. Schrenzel, G. Peters, and M. Herrmann. 2001b. Identification and characterization of a novel 38.5-kilodalton cell surface protein of *Staphylococcus aureus* with extended-spectrum binding activity for extracellualr matrix and plasma proteins. J. Bacteriol. **183:**6778-6786.

Hutchens, M., and G. D. Luker. 2007. Applications of bioluminescence imaging to the study of infectious diseases. Cell. Microbiol. **9:**2315-2322.

Jábes, D., G. Candiani, G. Romanó, C. Brunati, S. Riva, and M. Cavaleri. 2004. Efficacy of dalbavancin against methicillin-resistant *Staphylococcus aureus* in the rat granuloma pouch infection model. Antimicrob. Agents Chemother. **48:**1118-1123.

Jack, R. W., and H. G. Sahl. 1995. Unique peptide modifications involved in the biosynthesis of lantibiotics. Trends Biotechnol. **13:**269-278.

Jarvis, B., and J. Farr. 1971. Partial purification, specificity and mechanism of action of the nisin-inactivating enzyme from *Bacillus cereus*. Biochim. Biophys. Acta **227:**232-240.

Jawhara, S., and S. Mordon. 2004. *In vivo* imaging of bioluminescent *Escherichia coli* in a cutaneous wound infection model for evaluation of an antibiotic therapy. Antimicrob. Agents Chemother. **48:**3436-3441.

Jin, T., M. Bokarewa, T. Foster, J. Mitchell, J. Higgins, and A. Tarkowski. 2004. *Staphylococcus aureus* resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. J. Immunol. **172:**1169-1176.

Johanson, W. G., and L. L. Dever. 2003. Nosocomial pneumonia. Intensive Care Med. **29:**23-29.

Johnson, A. P., A. Pearson, and G. Duckworth. 2005. Surveillance and epidemiology of MRSA bacteraemia in the UK. J. Antimicrob. Chemother. **56:**455-462.

Johnston, N. J., T. A. Mukhtar, and G. D. Wright. 2002. Streptogramin antibiotics: mode of action and resistance. Curr. Drug Targets **3:**335-344.

Jones, H. A., S. Sriskandan, A. M. Peters, N. B. Pride, T. Krausz, A. R Boobis, and C. Haslett. 1997. Dissociation of neutrophil emigration and metabolic activity in lobar pneumonia and bronchiectasis. Eur. Respir. J. **10:**795-803.

Jurczok, A., P. Fornara, and A. Söling. 2008. Bioluminescence imaging to monitor bladder cancer cell adhesion *in vivo*: a new approach to optimize a syngeneic, orthotopic, murine bladder cancer model. BJU Int. **101:**120-124.

Kadurugamuwa, J. L., K. Modi, J. Yu, K. P. Francis, C. Orihuela, E. Tuomanen, A. F. Purchio, and P. R. Contag. 2005b. Noninvasive monitoring of pneumococcal meningitis and evaluation of treatment efficacy in an experimental mouse model. Mol. Imaging **4:**137-142.

Kadurugamuwa, J. L., K. Modi, J. Yu, K. P. Francis, T. Purchio, and P. R. Contag. 2005c. Noninvasive biophotonic imaging for monitoring of catheter-associated urinary tract infections and therapy in mice. Infect. Immun. **73:**3878-3887.

Kadurugamuwa, J. L., K. Modi, O. Coquoz, B. Rice, S. Smith, P. R. Contag, and T. Purchio. 2005a. Reduction of astrogliosis by early treatment of pneumococcal meningitis

measured by simultaneous imaging, *in vivo*, of the pathogen and host response. Infect. Immun. **73:**7836-7843.

Kadurugamuwa, J. L., L. Sin, E. Albert, J. Yu, K. Francis, M. DeBoer, M. Rubin, C. Bellinger-Kawahara, T. R. Parr, and P. R. Contag. 2003a. Direct continuous method for monitoring biofilm infection in a mouse model. Infect. Immun. **71:**882-890.

Kadurugamuwa, J. L., L. V. Sin, J. Yu, K. P. Francis, R. Kimura, T. Purchio, and P. R. Contag. 2003b. Rapid direct method for monitoring antibiotics in a mouse model of bacterial biofilm infection. Antimicrob. Agents Chemother. **47:**3130-3137.

Kadurugamuwa, J. L., L. V. Sin, J. Yu, K. P. Francis, T. F. Purchio, and P. R. Contag. 2004. Noninvasive optical imaging method to evaluate postantibiotic effects on biofilm infection *in vivo*. Antimicrob. Agents Chemother. **48:**2283-2287.

Kalmeijer, M. D., H. Coertjens, P. M. van Nieuwland-Bollen, D. Bogaers-Hofman, G. A. de Baere, A. Stuurman, A. van Belkum, and J. A. Kluytmans. 2002. Surgical site infections in orthopedic surgery: the effect of mupirocin nasal ointment in a double-blind, randomized, placebo-controlled study. Clin. Infect. Dis. **35:**353-358.

Kandulski, A., M. Selgrad, and P. Malfertheiner. 2008. *Helicobacter pylori* infection: a clinical review. **40:**619-626.

Kehoe, L. E., J. Snidwongse, P. Courvalin, J. B. Rafferty, and I. A. Murray. 2003. Structural basis of Synercid (quinupristin-dalfopristin) resistance in Gram-positive bacterial pathogens. J. Biol. Chem. **278:**29963-29970.

Kilian, K., and B. M. Kisiel. 2006. Pathogenesis and novel possibilities of diagnosis of cystic fibrosis. Pol. Merkur. Lekarski. **20:**582-585.

Kim, T. S., J. W. Hur, M. A. Yu, C. I. Cheigh, K. N. Kim, J. K. Hwang, and Y. R. Pyun. 2003. Antagonism of *Helicobacter pylori* by bacteriocins of lactic acid bacteria. J. Food Prot. **66:**3-12.

Kips, J. C., G. P. Anderson, J. J. Fredberg, U. Herz, M. D. Inman, M. Jordana, D. M. Kemeny, J. Lötvall, R. A. Pauwels, C. G. Plopper, D. Schmidt, P. J. Sterk, A. J. van Oosterhout, B. B. Vargaftig, and K. F. Chung. 2003. Murine models of asthma. Eur. Respir. J. **22:**374-382.

Kirmani, N., C. U. Tuazon, H. W. Murray, A. E. Parrish, and J. N. Sheagren. 1978. *Staphylococcus aureus* carriage rate of patients receiving long-term hemodialysis. Arch. Intern. Med. **138:**1657-1659.

Klaenhammer, T. R. 1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev. **12:**39-85.

Klastersky, J. 2003. Role of quinupristin/dalfopristin in the treatment of Gram-positive nosocomial infections in haematological or oncological patients. Cancer Treat. Rev. **29:**431-440.

Kokai-Kun, J. F., S. M. Walsh, T. Chanturiya, and J. J. Mond. 2003. Lysostaphin cream eradicates *Staphylococcus aureus* nasal colonization in a cotton rat model. Antimicrob. Agents Chemother. **47:**1589-1597.

Kola, A., P. Kirschner, B. Gohrbandt, I. F. Chaberny, F. Mattner, M. Strüber, P. Gastmeier, and S. Suerbaum. 2007. An infection with linezolid-resistant *S. aureus* in a patient with left ventricular assist system. Scand. J. Infect. Dis. **39:**463-465.

Kramer, N. E., S. A. van Hijum, J. Knol, J. Kok, and O. P. Kuipers. 2006. Transcriptome analysis reveals mechanisms by which *Lactococcus lactis* acquires nisin resistance. Antimicrob. Agents Chemother. **50:**1753-1761.

Kreikemeyer, B., D. McDevitt, and A. Podbielski. 2002. The role of the map protein in *Staphylococcus aureus* matrix protein and eukaryotic cell adherence. Int. J. Med. Microbiol. **292:**283-295.

Krinke, G.J. 2000. The laboratory rat. Elsevier Academic Press, London, UK.

Kruszewska, D., H. G. Sahl, G. Bierbaum, U. Pag, S. O. Hynes, and A. Ljungh. 2004. Mersacidin eradicates methicillin-resistant *Staphylococcus aureus* (MRSA) in a mouse rhinitis model. J. Antimicrob. Chemother. **54:**648-653.

Kuklin, N. A., G. D. Pancari, T. W. Tobery, L. Cope, J. Jackson, C. Gill, K. Overbye, K. P. Francis, J. Yu, D. Montgomery, A. S. Anderson, W. McClements, and K. U. Jansen. 2003. Real-time monitoring of bacterial infection *in vivo:* development of bioluminescent staphylococcal foreign-body and deep-thigh-wound mouse infection models. Antimicrob. Agents Chemother. **47:**2740-2748.

Kuraki, T., M. Ishibashi, M. Takayama, M. Shiraishi, and M. Yoshida. 2002. A novel oral neutrophil elastase inhibitor (ONO-6818) inhibits human neutrophil elastase-induced emphysema in rats. Am. J. Respir. Crit. Care Med. **166:**496-500.

Lauková, A., and S. Czikková. 2001. Antagonistic effect of enterocin CCM 4231 from *Enterococcus faecium* on "bryndza", a traditional Slovak dairy product from sheep milk. Microbiol. Res. **156:**31-34.

Leevy, W. M., S. T. Gammon, H. Jiang, J. R. Johnson, D. J. Maxwell, E. N. Jackson, M. Marquez, D. Piwnica-Worms, and B. D. Smith. 2006. Optical imaging of bacterial infection in living mice using a fluorescent near-infrared molecular probe. **128:**16476-16477.

Li, D., K. Gromov, K. Søballe, J. E. Puzas, R. J. O'Keefe, H. Awad, H. Drissi, and E. M. Schwarz. 2008. Quantitative mouse model of implant-associated osteomyelitis and the kinetics of microbial growth, osteolysis, and humoral immunity. J. Orthop. Res. **26:**96-105.

Lina, G., Y. Gillet, F. Vandenesch, M. E. Jones, D. Floret, and J. Etienne. 1997. Toxin involvement in staphylococcal scalded skin syndrome. Clin. Infect. Dis. **25:**1369-1373.

Lina, G., Y. Piémont, F. Godail-Gamot, M. Bes, M. O. Peter, V. Gauduchon, F. Vandenesch, and J. Etienne. 1999. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. **29:**1128-1132.

Line, J. E., E. A. Svetoch, B. V. Eruslanov, V. V. Perelygin, E. V. Mitsevich, I. P. Mitsevich, V. P. Levchuk, O. E. Svetoch, B. S. Seal, G. R. Siragusa, and N. J. Stern. 2008. Isolation and purification of enterocin E-760 with broad antimicrobial activity against Gram-positive and Gram-negative bacteria. Antimicrob. Agents Chemother. **52:**1094-1100.

Ling, T. K., K. S. Fung, and A. F. Cheng. 2001 *In vitro* activity and post-antibiotic effect of quinupristin/dalfopristin (Synercid). Chemotherapy **47:**243-249.

Lipsky, B. A., R. E. Pecoraro, M. S. Chen, and T. D. Koepsell. 1987. Factors affecting staphylococcal colonization among NIDDM outpatients. Diabetes Care **10:**483-486.

Lloyd, A. G., and J. J. Drake. 1975. Problems posed by essential food preservatives. Br. Med. Bull. **31:**214-219.

Lorenz, W. W., R. O. McCann, M. Longiaru, and M. J. Cormier. 1991. Isolation and expression of a cDNA encoding *Renilla reniformis* luciferase. Proc. Natl. Acad. Sci. U.S.A. **88:**4438-4442.

Lowy, F. D. 1998. *Staphylococcus aureus* infections. N. Engl. J. Med. **339:**520-532.

Lozo, J., M. Vukasinovic, I. Strahinic, and L. Topisirovic. 2004. Characterization and antimicrobial activity of bacteriocin 217 produced by natural isolate *Lactobacillus paracasei* subsp. *paracasei* BGBUK2-16. J. Food Prot. **67:**2727-2734.

Luo, J., A. H. Lin, E. Masliah, and T. Wyss-Coray. 2006. Bioluminescence imaging of Smad signaling in living mice shows correlation with excitotoxic neurodegeneration. Proc. Natl. Acad. Sci. U.S.A. **103:**18326-18331.

Machida, K. 1995. Immunological investigations on pathogenesis of staphylococcal scalded skin syndrome. Rinsho Byori. **43:**547-556.

Mack, D., P. Becker, I. Chatterjee, S. Dobinsky, J. K. M. Knobloch, G. Peters, H. Rohde, and M. Herrmann. 2004. Mechanisms of biofilm formation in *Staphylococcus epidermidis*

and *Staphylococcus aureus*: functional molecules, regulatory circuits and adaptive responses. Int. J. Med. Microbiol. **294:**203-212.

Maisnier-Patin, S., and J. Richard. 1996. Cell wall changes in nisin-resistant variants of *Listeria innocua* grown in the presence of high nisin concentrations. FEMS Microbiol. Lett. **140:**29-35.

Mangili, A., I. Bica, D. R. Snydman, and D. H. Hamer. 2005. Daptomycin-resistant, methicillin-resistant *Staphylococcus aur*eus bacteremia. **40:**1058-1060.

Mantovani, H. C., and J. B. Russell. 2001. Nisin resistance of *Streptococcus bovis*. Appl. Environ. Microbiol. **67:**808-813.

Marques, C. N., V. C. Salisbury, J. Greenman, K. E. Bowker, and S. M. Nelson. 2005. Discrepancy between viable counts and light output as viability measurements, following ciprofloxacin challenge of self-bioluminescent *Pseudomonas aeruginosa* biofilms. J. Antimicrob. Chemother. **56:**665-671.

Marty, F. M., W. W. Yeh, C. B. Wennersten, L. Venkataraman, E. Albano, E. P. Alyea, H. S. Gold, L. R. Baden, and S. K. Pillai. 2006. Emergence of clinical daptomycin-resistant *Staphylococcus aureus* isolate during treatment of methicillin-resistant *Staphylcoccus aureus* bacteremia and osteomyelitis. J. Clin. Microbiol. **44:**595-597.

Mattick, A. T. R., and A. Hirsch. 1947. Further observation on an inhibitory substance (nisin) from lactic streptococci. Lancet **ii:**5-12.

Mazzotta, A. S., and T. J. Montville. 1997. Nisin induces changes in membrane fatty acid composition of *Listeria monocytogenes* nisin-resistant strains at 10 degrees C and 30 degrees C. J. Appl. Microbiol. **82:**32-38.

Medzhitov, R., and C. Jr. Janeway. 2000. Innate immunity. N. Engl. J. Med. **343:**338-344.

Meighen, E. A. 1993. Bacterial bioluminescence: organization, regulation, and application of the *lux* genes. FASEB J. **7:**1016-1022.

Meighen, E. A. 1994. Genetics of bacterial bioluminescence. Annu. Rev. Genet. **28:**117-139.

Meyers, B. R. 1984. Bacterial sinusitis. J. Fam. Pract. **18:**117-118.

Micek, S. T., K. E. Kollef, R. M. Reichley, N. Roubinian, and M. H. Kollef. 2007. Health care-associated pneumonia and community-acquired pneumonia: a single center experience. Antimicrob. Agents Chemother. **51:**3568-3573.

Miller, L. G., F. Perdreau-Remington, G. Rieg, S. Mehdi, J. Perlroth, A. S. Bayer, A. W. Tang, T. O. Phung, and B. Spelberg. 2005. Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angelos. N. Engl. J. Med. **352:**1445-1453.

Moellering, R. C., J. R. Graybill, J. E. McGowan, and L. Corey. 2007. Antimicrobial resistance prevention initiative – an update: proceedings of an expert panel on resistance. Am. J. Med. **120:**S4-S25.

Morency, H., M. Mota-Meira, G. LaPointe, C. Lacroix, and M. C. Lavoie. 2001. Comparison of the activity spectra against pathogens of bacterial strains producing a mutacin or a lantibiotic. Can. J. Microbiol. **47:**322-331.

Mortin, L. I., T. Li, A. D. van Praagh, S. Zhang, X.-X. Zhang, and J. D. Alder. 2007. Rapid bactericidal activity of daptomycin against methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* peritonitis in mice as measured with bioluminescent bacteria. Antimicrob. Agents Chemother. **51:**1787-1794.

Mulders, J. W., I. J. Boerrigter, H. S. Rollema, R. J. Siezen, and W. M. de Vos. 1991. Identification and characterization of the lantibiotic nisin Z, a natural nisin variant. Eur. J. Biochem. **201:**581-584.

Müller, D. M., M. S. Carrasco, A. C. Simonetta, L. M. Beltramini, and G. G. Tonarelli. 2007. A synthetic analog of plantaricin 149 inhibiting food-borne pathogenic bacteria: evidence for alpha-helical conformation involved in bacteria-membrane interaction. J. Pept. Sci. **13:**171-178.

Naimi, T. S., K. H. LeDell, K. Como-Sabetti, S. M. Borchardt, D. J. Boxrud, J. Etienne, S. K. Johnson, F. Vandenesch, S. Fridkin, C. O'Boyle, R. N. Danila, and R. Lynfield. 2003. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA **290:**2976-2984.

Nashev, D., K. Toshkova, S. I. Salasia, A. A. Hassan, C. Lämmler, and M. Zschöck. 2004. Distribution of virulence genes of *Staphylococcus aureus* isolated from stable nasal carriers. FEMS Microbiol. Lett. **233:**45-52.

Nissen-Meyer, J., H. Holo, L. S. Håvarstein, K. Sletten, and I. F. Nes. 1992. A novel lactococcal bacteriocin whose activity depends on the complementary action of two peptides. J. Bacteriol. **174:**5686-5692.

Nouwen, J., J. Schouten, P. Schneebergen, S. Snijders, J. Maaskant, M. Koolen, A. van Belkum, and H. A. Verbrugh. 2006. *Staphylococcus aureus* carriage patterns and the risk of infections associated with continuous peritoneal dialysis. J. Clin. Microbiol. **44:**2233-2236.

O'Brien, L. M., E. J. Walsh, R. C. Massey, S. J. Peacock, and T. J. Foster. 2002. *Staphylococcus aureus* clumping factor B (ClfB) promotes adherence to human type I cytokeratin 10: implications for nasal colonization. Cell. Microbiol. **4:**759-770.

Ogata, N., and T. Shibata. 2008. Protective effect of low-concentration chlorine dioxide gas against influenza A virus infection. J. Gen. Virol. **89:**60-67.

Orihuela, C. J., G. Gao, K. P. Francis, J. Yu, and E. I. Tuomanen. 2004. Tissue-spesific contributions of pneumococcal virulence factors to pathogenesis. J. Infect. Dis. **190:**1661-1669.

Pan, A., S. Lorenzotti, and A. Zoncada. 2008. Registered and investigational drugs for the treatment of methicillin-resistant *Staphylococcus aureus* infection. Recent Patents Anti-Infect Drug Disc. **3:**10-33.

Patradoon-Ho, P., and D. A. Fitzgerald. 2007. Lung abscess in children. Paediatr. Respir. Rev. **8:**77-84.

Pattanayak, S. P., and P. Sunita. 2008. Wound healing, antimicrobial and antioxidant potential of *Dendrophthoe falcate* (L.f) Ettingsh. J. Ethnopharmacol. **120:**241-247.

Peacock, S. J., A. Justice, D. Griffiths, G. D. de Silva, M. N. Kantzanou, D. Crook, K. Sleeman, and N. P. Day. 2003. Determinants of acquisition and carriage of *Staphylococcus aureus* in infancy. J. Clin. Microbiol. **41:**5718-5725.

Pépin, J., L. Valiquette, and B. Cossette. 2005. Mortality attributable to nosocomial *Clostridium difficile*-associated disease during an epidemic caused by a hypervirulent strain in Quebec. CMAJ **173:**1037-1042.

Peschel, A. 2002. How do bacteria resist human antimicrobial peptides? Trends Microbiol. **10:**179-186.

Peschel, A., M. Otto, R. W. Jack, H. Kalbacher, G. Jung, and F. Götz. 1999. Inactivation of the *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J. Biol. Chem. **274:**8405-8410.

Peschel, A., R. W. Jack, M. Otto, L. V. Collins, P. Staubitz, G. Nicholson, H. Kalbacher, W. F. Nieuwenhuizen, G. Jung, A. Tarkowski, K. P. van Kessel, and J. A. van Strijp. 2001. *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with l-lysine. J. Exp. Med. **193:**1067-1076.

Piva, A., and D. R. Headon. 1994. Pediocin A, a bacteriocin produced by *Pediococcus pentosaceus* FBB61. Microbiology **140:**697-702.

Piwnica-Worms, D., D. P. Schuster, and J. R. Garbow. 2004. Molecular imaging of host-pathogen interactions in intact small animals. Cell. Microbiol. **6:**319-331.

Poindexter, N. J., and P. M. Schlievert. 1986. Suppression of immunoglobulin-secreting cells from human peripheral blood by toxic-shock-syndrome toxin-1. J. Infect. Dis. **153:**772-779.

Prasad, K. M., Y. Xu, Z. Yang, M. C. Toufektsian, S. S. Berr, and B. A. French. 2007. Topoisomerase inhibition accelerates gene expression after adeno-associated virus-mediated gene transfer to the mammalian heart. Mol. Ther. **15:**764-771.

Pujol, M., X. Corbella, C. Peña, R. Pallares, J. Dorca, R. Verdaguer, A. Diaz-Prieto, J. Ariza, and F. Gudiol. 1998. Clinical and epidemiological findings in mechanically-ventilated patients with methicillin-resistant *Staphylococcus aureus* pneumonia. Eur. J. Clin. Microbiol. Infect. Dis. **17:**622-628.

Quesada, I., C. Villalobos, L. Núñez, P. Chamero, M. T. Alonso, A. Nadal, and J. García-Sancho. 2008. Glucose induces synchronous mitochondria calcium oscillations in intact pancreatic islets. Cell. Calcium **43:**39-47.

Ramsey, A. M. 2002. Diagnosis and treatment of the child with a draining ear. J. Pediatr. Health Care **16:**161-169.

Rauch, P. J., and W. M. de Vos. 1992. Characterization of the novel nisin-sucrose conjugative transposon Tn5276 and its insertion in *Lactococcus lactis.* J. Bacteriol. **174:**1280-1287.

Rea, M. C., E. Clayton, P. M. O'Connor, F. Shanahan, B. Kiely, R. P. Ross, and C. Hill. 2007. Antimicrobial activity of lacticin 3147 against clinical *Clostridium difficile* strains. J. Med. Microbiol. **56:**940-946.

Reddy, K. V., C. Aranha, S. M. Gupta, and R. D. Yedery. 2004. Evaluation of antimicrobial peptide nisin as a safe vaginal contraceptive agent in rabbits: *in vitro* and *in vivo* studies. Reproduction **128:**117-126.

Ridder-Schaphorn, S., F. Ratjen, A. Dübbers, J. Häberle, S. Falk, P. Küster, A. Schuster, U. Mellies, B. Löwe, R. Reintjes, G. Peters, and B. C. Kahl. 2007. Nasal *Staphylococcus aureus* carriage is not a risk factor for lower-airway infection in young cystic fibrosis patients. J. Clin. Microbiol. **45:**2979-2984.

Rilla, N., B. Martínez, and A. Rodríquez. 2004. Inhibition of a methicillin-resistant *Staphylococcus aureus* strain in Afuega'l Pitu cheese by the nisin Z-producing strain *Lactococcus lactis* subsp. *lactis* IPLA 729. J. Food Prot. **67:**928-933.

Riordan, J. R., J. M. Rommens, B. Kerem, N. Alon, R. Rozmahel, Z. Grzelczak, J. Zielenski, S. Lok, N. Plavsic, and J. L. Chou. 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science **245:**1066-1073.

Rocchetta, H. L., C. J. Boylan, J. W. Foley, P. W. Iverson, D. L. LeTourneau, C. L. McMillian, P. R. Contag, D. E. Jenkins, and T. R. Parr. 2001. Validation of a noninvasive, real-time imaging technology using bioluminescent *Escherichia coli* in the neutropenic mouse thigh model of infection. Antimicrob. Agents Chemother. **45:**129-137.

Roth, D. J., E. D. Jansen, A. C. Powers, and T. G. Wang. 2006. A novel method of monitoring response to islet transplantation: bioluminescent imaging of an NF-kB transgenic mouse model. Transplantation **81:**1185-1190.

Ruef, C. 2004. Epidemiology and clinical impact of glycopeptide resistance in *Staphylococcus aureus*. Infection. **32:**315-327.

Ryan, M. P., J. Flynn, C. Hill, R. P. Ross, and W. J. Meaney. 1999. The natural food grade inhibitor, lacticin 3147, reduced the incidence of mastitis after experimental challenge with *Streptococcus dysgalactiae* in nonlactating dairy cows. J. Dairy Sci. **82:**2625-2631.

Sadikot, R. T., and T. S. Blackwell. 2005. Bioluminescence imaging. Proc. Am. Thorac. Soc. **2:**537-540.

Saiman, L. 2004. Microbiology of early CF lung disease. Paediatr. Respir. Rev. **5** Suppl. A S367-S369.

Sanderson, A. R., J. G. Leid, and D. Hunsaker. 2006. Bacterial biofilms on the sinus mucosa of human subjects with chronic rhinosinusitis. Laryngoscope **116:**1121-1126.

Scarff, J. M., and J. B. Goldberg. 2008. Vaccination against *Pseudomonas aeruginosa* pneumonia in immunocompromised mice. Clin. Vaccine Immunol. **15:**367-375.

Schleifer, K. H. 1987. Recent changes in the taxonomy of lactic acid bacteria. FEMS Microbiol. Lett. **46:**201-203.

Schlievert, P. M., L. C. Case, K. L. Strandberg, B. B. Abrams, and D. Y. Leung. 2008. Superantigen profile of *Staphylcoccus aureus* isolates from patients with steroid-resistant atopic dermatitis. Clin. Infect. Dis. **46:**1562-1567.

Schuster, D., J. Kozlowski, and L. Hogue. 2003. Imaging lung inflammation in a murine model of *Pseudomonas* infection: a positron emission tomography study. Exp. Lung Res. **29:**45-47.

Seeger, W., R. G. Birkemeyer, L. Erment, N. Suttorp, S. Bhakdi, and H. R. Duncker. 1990. Staphylococcal alpha-toxin-induced vascular leakage in isolated perfused rabbit lungs. Lab. Invest. **63:**341-349.

Severina, E., A. Severin, and A. Tomasz. 1998. Antibacterial efficacy of nisin against multidrug-resistant Gram-positive pathogens. J. Antimicrob. Chemother. **41:**341-347.

Shah, K., and R. Weissleder. 2005. Molecular optical imaging: applications leading to the development of present day therapeutics. NeuroRx **2:**215-225.

Shaner, N. C., P. A. Steinbach, and R. Y. Tsien. 2005. A guide to choosing fluorescent proteins. Nat. Methods **2:**905-909.

Siegers, K., and K. D. Entian. 1995. Genes involved in immunity to the lantibiotic nisin produced by *Lactococcus lactis* 6F3. Appl. Environ. Microbiol. **61:**1082-1089.

Silkin, L., S. Hamza, S. Kaufman, S. L. Cobb, and J. C. Vederas. 2008. Spermicidal bacteriocins: lacticin 3147 and subtilosin A. Bioorg. Med. Chem. Lett. **18:**3103-3106.

Silverman, J. A., N. G. Perlmutter, and H. M. Shapiro. 2003. Correlation of daptomycin bactericidal activity and membrane depolarization in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **47:**2538-2544.

Sissolak, D., A. Geusau, G. Heinze, W. Witte, and M. L. Rotter. 2002. Risk factors for nasal carriage of *Staphylococcus aureus* in infectious disease patients, including patients infected with HIV, and molecular typing of colonizing strains. Eur. J. Clin. Microbiol. Infect. Dis. **21:**88-96.

Sleator, R. D., and C. Hill. 2008. Designer probiotics: a potential for *Clostridium difficile*? J. Med. Microbiol. 57:793-794.

Smith, K., A. Perez, G. Ramage, D. Lappin, C. G. Gemmell, and S. Lang. 2008. Biofilm formation by Scottish clinical isolates of *Staphylococcus aureus*. J. Med. Microbiol. **57:**1018-1023.

Söderquist, B., I. Alriksson, J. Källman, and E. Kihlström. 2006. The influence of adhesive and invasive properties of *Staphylococcus aureus* defective in fibronectin-binding proteins on secretion of interleukin-6 by human endothelial cells. APMIS **114:**112-116.

Solberg, C. O. 2000. Spread of *Staphylococcus aureus* in hospitals: causes and prevention. Scand. J. Infect. Dis. **32:**587-595.

Somner, E. A., and P. E. Reynolds. 1990. Inhibition of peptidoglycan biosynthesis by ramoplanin. Antimicrob. Agents Chemother. **34:**413-419.

Spond, J., N. Case, R. W. Chapman, Y. Crawley, R. W. Egan, J. Fine, J. A. Hey, W. Kreutner, T. Kung, P. Wang, and M. Minnicozzi. 2003. Inhibition of experimental acute pulmonary inflammation by pirfenidone. Pulm. Pharmacol. Ther. **16:**207-214.

Stanaway, S., D. Johnson, P. Moulik, and G. Gill. 2007. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolation from diabetic foot ulcers correlates with nasal MRSA carriage. Diabetes Res. Clin. Pract. **75:**47-50.

Stearne, L. E., R. M. Schiffelers, E. Smouter, I. A. Bakker-Woudenberg, and I. C. Gyssens. 2002. Biodistribution of long-circulating PEG-liposomes in a murine model of established subcutaneous abscesses. Biochim. Biophys. Acta. **1561:**91-97.

Stein, T., S. Heinzmann, I. Solovieva, and K. D. Entian. 2003. Function of *Lactococcus lactis* nisin immunity genes *nisI* and *nisFEG* after coordinated expression in the surrogate host *Bacillus subtilis*. J. Biol. Chem. **278:**89-94.

Stone, A., and L. Saiman. 2007. Update on the epidemiology and management of *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus*, in patients with cystic fibrosis. Curr. Opin. Pulm. Med. **13:**515-521.

Stryjewski, M. E., W. D. O'Riordan. W. K. Lau, F. D. Pien, L. M. Dunbar, M. Vallee, V. G. Jr Fowler, V. H. Chu, E. Spencer, S. L. Barriere, M. M. Kitt, C. H. Cabell, G. R. Corey, and FAST Investigator Group. 2005. Telavancin versus standard therapy for treatment of complicated skin and soft-tissue infections due to Gram-positive bacteria. Clin. Infect. Dis. **40:**1601-1607.

Szittner, R., and E. A. Meighen. 1990. Nucleotide sequence, expression, and properties of luciferase coded by *lux* genes from a terrestrial bacterium. J. Biol. Chem. **265:**16581-16587.

Tagg, J. R., A. S. Dajani, and L. W. Wannamaker. 1976. Bacteriocins of Gram-positive bacteria. Bacteriol. Rev. **40:**722-756.

Tarner, I. H., A. J. Slavin, J. McBride, A. Levicnik, R. Smith, G. P. Nolan, C. H. Contag, and C. G. Fathman. 2003. Treatment of autoimmune disease by adoptive cellular gene therapy. Ann. N.Y. Acad. Sci. **998:**512-519.

Thal, L. A., and M. J. Zervos. 1999. Occurrence and epidemiology of resistance to virginiamycin and streptogramins. J. Antimicrob. Chemother. **43:**171-176.

Todorov, S. D., and L. M. Dicks. 2004. Characterization of mesentericin ST99, a bacteriocin produced by *Leuconostoc mesenteroides* subsp. *dextranicum* ST99 isolated from boza. J. Ind. Microbiol. Biotechnol. **31:**323-329.

Todorov, S., B. Onno, O. Sorokine, J. M. Chobert, I. Ivanova, and X. Dousset. 1999. Detection and characterization of a novel antibacterial substance produced by *Lactobacillus plantarum* ST31 isolated from sourdough. Int. J. Food Microbiol. **48:**167-177.

Tolar, J., X. Wang, E. Braunlin, R. T. McElmurry, Y. Nakamura, S. Bell, L. Xia, J. Zhang, Q. Hu, A. Panoskaltasis-Mortari, J. Zhang, and B. R. Blazar. 2007. The host immune response is essential for the beneficial effect of adult stem cells after myocardial ischemia. Exp. Hematol. **35:**682-690.

Ulitzur, S. 1997. Established technologies and new approaches in applying luminous bacteria for analytical purposes. J. Biolumin. Chemilumin **12:**179-192.

Ulrich, M., S. Herbert, J. Berger, G. Bellon, D. Louis, G. Münker, and G. Döring. 1998. Localization of *Staphylococcus aureus* in infected airways of patients with cystic fibrosis and in a cell culture model of *S. aureus* adherence. Am. J. Respir. Cell Mol. Biol. **19:**83-91.

Ugurlu, T., M. Turkoglu, U. S. Gurer, and B. G. Akarsu. 2007. Colonic delivery of compression coated nisin tablets using pectin/HPMC polymer mixture. Eur. J. Pharm. Biopharm. **67:**202-210.

Valenta, C., A. Bernkop-Schnürch, and H. P. Rigler. 1996. The antistaphylococcal effect of nisin in a suitable vehicle: a potential therapy for atopic dermatitis in man. J. Pharm. Pharmacol. **48:**988-991.

van Bambeke, F. 2006. Glycopeptides and glycodepsipeptides in clinical development: a comparative review of their antibacterial spectrum, pharmacokenetics and clinical efficacy. Curr. Opin. Investig. Drugs **7:**740-749.

van Heusden, H. E., B. de Kruijff, and E. Breukink. 2002. Lipid II induces a transmembrane orientation of the pore-forming peptide lantibiotic nisin. Biochemistry **41:**12171-12178.

Vannuffel, P., and C. Cocito. 1996. Mechanism of action of streptogramins and macrolides. **51:**20-30.

Vautor, E., G. Abadie, J. M. Guibert, N. Chevalier and M. Pépin. 2005. Nasal carriage of *Staphylococcus aureus* in dairy sheep. Vet. Microbiol. **106:**235-239.

Verhoeff, M., E. L. van der Veen, M. M. Rovers, E. A. Sanders, and A. G. Schilder. 2006. Chronic suppurative otitis media: a review. Int. J. Pediatr. Otorhinolaryngol. **70:**1-12.

Wachsmans, M. B., M. E. Farías, E. Takeda, F. Sesma, A. P. de Ruiz Holgado, R. A. de Torres, and C. E. Coto. 1999. Antiviral activity of enterocin CRL35 against herpesviruses. Int. J. Antimicrobial. Agents **12:**293-299.

Wachsmans, M. B., V. Castilla, A. P. de Ruiz Holgado, R. A. de Torres, F. Sesma, and C. E. Coto. 2003. Enterocin CRL35 inhibits late stages of HSV-1 and HSV-2 replication *in vitro*. Antiviral Res. **58:**17-24.

Wang, X., Y. Liu, B. Xie, X. Shi, J. Zhou, and H. Zhang. 2005. Effect of nisin on the growth of *Staphylococcus aureus* determined by a microcalorimetric method. Mol. Nutr. Food Res. **49:**350-354.

Wan, X. Y., L. Y. Bi, and Y. L. Zhang. 2006. Protective effect of glutamine on rats with pseudomonas pneumonia. Zhonghua Nei Ke Za Zhi **45:**1004-1007.

Weidenmaier, C., J. F. Kokai-Kun, S. A. Kristian, T. Chanturiya, H. Kalbacher, M. Gross, G. Nicholson, B. Neumeister, J. J. Mond, and A. Peschel. 2004. Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. Nat. Med. **10:**243-245.

Weigel, L. M., R. M. Donlan, D. H. Shin, B. Jensen, N. C. Clark, L. K. McDougal, W. Zhu, K. A. Musser, J. Thompson, D. Kohlerschmidt, N. Dumas, R. J. Limberger, and J. B. Patel. 2007. High-level vancomycin-resistant *Staphylococcus aureus* isolates associated with a polymicrobial biofilm. Antimicrob. Agents Chemother. **51:**231-238.

Wertheim, H. F., D. C. Melles, M. C. Vos, W. van Leeuwen, A. van Belkum, H. A. Verbrugh, and J. L. Nouwen. 2005. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect. Dis. **5:**751-762.

Wertheim, H. F., M. C. Vos, A. Ott, A. Voss, J. A. Kluytmans, C. M. Vandenbroucke-Grauls, M. H. Meester, P. H. van Keulen, and H. A. Verbrugh. 2004. Mupirocin prophylaxis against nosocomial *Staphylococcus aureus* infections in nonsurgical patients: a randomized study. Ann. Intern. Med. **140:**419-425.

WHO Expert Committee on Biological Standardization. 1969. Twenty-second report, WHO Technical Report Series No. 444.

Wiedemann, I., E. Breukink, C. van Kraaij, O. P. Kuipers, G. Bierbaum, B. de Kruijff, and H. G. Sahl. 2001. Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. J. Biol. Chem. **276:**1772-1779.

Williams, J. V., B. R. Vowels, P. J. Honig, and J. J. Leyden. 1998. *Staphylococcus aureus* isolation from the lesions, the hands, and the anterior nares of patients with atopic dermatitis. Pediatr. Dermatol. **15:**194-198.

Wirawan, R. E., N. A. Klesse, R. W. Jack, and J. R. Tagg. 2006. Molecular and genetic characterization of a novel nisin variant produced by *Streptococcus uberis*. Appl. Environ. Microbiol. **72:**1148-1156.

Wu, J., S. Hu, and L. Cao. 2007. Therapeutic effect of nisin Z on subclinical mastitis in lactating cows. Antimicrob. Agents Chemother. **51:**3131-3135.

Xi, L., K. W. Cho, and S. C. Tu. 1991. Cloning and nucleotide sequence of *lux* genes and characterization of luciferase of *Xenorhabdus luminescens* from a human wound. J. Bacteriol. **173:**1399-1405.

Xiong, Y. Q., J. Willard, J. L. Kadurugamuwa, J. Yu, K. P. Francis, and A. S. Bayer. 2005. Real-time *in vivo* bioluminescent imaging for evaluating the efficacy of antibiotics in a rat *Staphylococcus aureus* endocarditis model. Antimicrob. Agents Chemother. **49:**380-387.

Yarwood, J. M., K. M. Paquette, I. B. Tikh, E. M. Volper, and E. P. Greenberg. 2007. Generation of virulence factor variants in *Staphylococcus aureus* biofilms. J. Bacteriol. **189:**7961-7967.

Yu, J., J. Wu, K. P. Francis, T. F. Purchio, and J. L. Kadurugamuwa. 2005. Monitoring *in vivo* fitness of rifampicin-resistant *Staphylococcus aureus* mutants in a mouse biofilm infection model. J. Antimicrob. Chemother. **55:**528-534.

Zendo, T., M. Fukao, K. Ueda, T. Higuchi, J. Nakayama, and K. Sonomoto. 2003. Identification of the lantibiotic nisin Q, a new natural nisin variant produced by *Lactococcus lactis* 61-14 isolated from a river in Japan. Biosci. Biotechnol. Biochem. **67:**1616-1619.

Zhao, H., R. J. Wong, X. Nguyen, F. Kalish, M. Mizobuchi, H. J. Vreman, D. K. Stevenson, and C. H. Contag. 2006. Expression and regulation of heme oxygenase isozymes in the developing mouse cortex. Pediatr. Res. **60:**518-523.

Chapter 3

Characterization of the structural gene encoding nisin F, a new lantibiotic produced by *Lactococcus lactis* subsp. *lactis* isolate from freshwater catfish (*Clarias gariepinus*)

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Lactococcus lactis F10, isolated from freshwater catfish, produces a bacteriocin (BacF) active against *Staphylococcus aureus, Staphylococcus carnosus, Lactobacillus curvatus, Lactobacillus plantarum* and *Lactobacillus reuteri*. The operon encoding BacF is located on a plasmid. Sequencing of the structural gene revealed homology to other nisin genes. Nisin F is described.

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Lantibiotics are small, ribosomally synthesized peptides with lanthionine rings and dehydrated amino acid residues. Nisin A was isolated from a strain of *Lactococcus lactis* subsp. *lactis* by Mattick and Hirsch (14), and the structure was published by Gross and Morell (10). Since then, a number of lantibiotics have been described, mostly from *Lactococcus lactis*, *Streptococcus*, *Bacillus*, *Staphylococcus,* and *Streptomyces* spp. (6).

The nisin A operon has been studied in detail and consists of 11 genes, i.e. *nisA, nisB, nisT, nisC, nisI, nisP, nisR, nisK, nisF, nisE,* and *nisG.* The prepeptide is encoded by the structural gene *nisA* (2). *NisB* and *nisC* are involved in maturation of the lantibiotic, and *nisT* is involved in transport across the cell membrane (7). *NisI* encodes an immunity protein, and *nisP* encodes a putative serine protease involved in processing (8). *NisR* and *nisK* encode a putative regulatory protein and a putative histidine kinase, respectively (8, 20). *NisF*, *nisE* and *nisG* encode ATP-binding cassette transporters that are, together with *nisI*, responsible for immunity (18). The bacteriocin described in this paper is structurally different from nisins A, Z, Q, U and U2 and represents a sixth variation of the lantibiotic, designated nisin F.

Lactic acid bacteria (LAB) were cultured in De Man Rogosa and Sharpe (MRS) broth (Biolab, Biolab Diagnostics, Midrand, South Africa) at 30°C, *Staphylococcus aureus* was cultured in brain heart infusion (BHI, Biolab) at 37°C, and *Escherichia coli* DH5α in Luria Bertani broth (Biolab) on a rotating wheel (model TC-7; New Brunswick Scientific Co., Inc., Edison, NJ) at 37°C. The target strains and growth conditions are listed in Table 1.

Feces collected from the intestinal tract of *Clarias gariepinus,* a freshwater catfish from a river in Stellenbosch, South Africa, were inoculated into MRS broth and incubated for 24 h at 30ºC. Cultures were streaked onto MRS agar (Biolab) plates and incubated for 24 h at 30ºC. Plates with fewer than 50 colonies were overlaid with BHI soft agar, each containing a pure culture of *S. aureus.* Colonies of lactic acid bacteria with the largest inhibition zones were selected and purified by streaking onto MRS agar. The presence of antimicrobial compounds in cell-free culture supernatants was confirmed by using the agarspot method (12).

The strain that showed the best antimicrobial activity was selected and identified according to sugar fermentation reactions (API 50 CHL; bioMérieux, France), key differential characteristics (11) and 16S rRNA gene sequencing (9).

Bacteriocin F was semipurified by ammonium sulphate precipitation and dialysis according to the method described by Sambrook et al. (16). The stability of the semipurified

bacteriocin against different enzymes, temperatures and pH levels was tested according to the method of Todorov and Dicks (19). The activity of samples was tested by using the agarspot method (12). The antimicrobial activity of the crude bacteriocin was tested against *S. aureus* and strains from the Laboratorium voor Microbiologie (LMG) panel (Table 1). Each strain (1 x 10^6 CFU ml^{-1}) was embedded in soft agar and the activity of nisin was tested as described before. The approximate molecular size of the BacF was determined by Tricine-sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (17), as described by Van Reenen et al. (21). *S. aureus* K (10^6 CFU ml^{-1}) was used as a sensitive strain.

Strain F10 was cured from plasmids according to the method of de Kwaadsteniet et al. (4), modified by supplementing MRS broth with 20 to 80 µg ml^{-1} ethidium bromide (Sigma) instead of novobiocin or SDS.

Genomic and plasmid DNA of strain F10 was isolated according to the method described by Dellaglio et al. (5) and by using a plasmid midi kit (Qiagen Inc., Valencia, CA), respectively. The size of the plasmid was determined from fragments generated by digesting with AccI (Boehringer Mannheim, GmbH, Germany), as described by Sambrook et al. (16).

Genomic and plasmid DNA was amplified with primers designed from the structural gene of nisin Q (GenBank accession number AB100029). The following primers were used: nisin forward primer (nisF), 5-'ATGAGTACAAAAGATTTCAACTT-3', and nisin reverse primer (nisR), 5'-TTATTTGCTTACGTGAACGC-3'. The amplification conditions were as follows: 1 cycle of 94°C for 4 min; 35 cycles of 94°C for 1 min, 48°C for 30 s and 72°C for 7 s; and 1 cycle of 72°C for 7 min. PCR products were cleaned with QIAquick PCR purification kit (Qiagen) and sequenced with an ABI Prism TM377 DNA Sequencer (PE Biosystems SA). The exact molecular size of mature BacF (without leader peptide) was determined from the amino acid sequence deduced from the DNA sequence. DNA homology to sequences listed in GenBank was determined using the BLAST program (1).

Sequencing of the 16S rRNA gene PCR product revealed 99% homology between strain F10, *Lactococcus lactis* subsp. *lactis* IL1403 and *L. lactis* strain KLDS 4.0319. Strain F10 is thus classified as *L. lactis* subsp. *lactis*. BacF inhibited the growth of two strains of *S. aureus* that have been isolated from patients diagnosed with sinusitis, *Lactobacillus curvatus* LMG 13553, *Lactobacillus plantarum* LMG 13556, *Lactobacillus reuteri* LMG 13557 and *Staphylococcus carnosus* LMG 13567 (Table 1). Antimicrobial activity was lost after treatment with pronase,

confirming that activity was conferred by a peptide. The bacteriocin remained active after treatment with amylase, pepsin and proteinase K. Levels of antimicrobial activity remained unchanged after 100 min at 90°C, but activity was lost after autoclaving. Activity was recorded over a broad pH range (from pH 2-10). According to SDS-polyacrylamide gel electrophoresis, the bacteriocin is between 6.5 and 14.3 kDa in size (Fig. 1). The size and the isoelectric point of the mature peptide (without the leader peptide) is respectively 3,457 kDa and 8.73, as deduced from the DNA sequence of the structural gene encoding the mature peptide.

As far as we could determine, this is the second report of bacteriocin-producing strains of *L. lactis* isolated from fresh fish. The paper by Campos et al. (3) described strains of *L. lactis* with activity against *Listeria monocytogenes* and *S. aureus*, but did not characterize the bacteriocins.

L. lactis F10 harbours a plasmid of approximately 24 kb. Strain F10 lost the ability to produce bacteriocin F after growth in the presence of ethidium bromide (30 µg ml^{-1}), suggesting that the genes encoding the bacteriocin are located on the plasmid. The DNA primers that we have designed differ by one or two bases from the structural genes of nisin A, nisin Z and nisin U (not shown). The sequence of the DNA fragment amplified from strain F10 (GenBank accession number EU057979) is similar to the sequence recorded for the structural gene encoding nisin Z (GenBank accession number X61144) (15), except for valine instead of isoleucine at position 30. Homology was also recorded with nisin Q (GenBank accession number AB100029) (36), also having valine at position 30 (Fig. 2). However, bacF differs from nisin Q by having alanine at position 15 and not valine, and methionine at position 21 instead of leucine. It is interesting to note that nisin Q was also isolated from a river in Japan (22). Bacteriocin F is thus regarded as a new variety of nisin and is named nisin F.

Nisin F proved very effective against clinical strains of *S. aureus* and may be used to prevent sinusitis. We are at present conducting research on Wistar rats to test this hypothesis. In previous studies, the lantibiotic mersacidin has been used to eradicate *S. aureus* from the respiratory tract of mice (13).

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REFERENCES

1. **Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389-3402.
2. **Buchman, G. W., S. Banerjee, and J. N. Hansen.** 1988. Structure, expression, and evolution of a gene encoding the precursor of nisin, a small protein antibiotic. J. Biol. Chem. **263:**16260-16266.
3. **Campos, C. A., Ó. Rodríquez, P. Calo-Mata, M. Prado, and J. Barros-Velázquez.** 2006. Preliminary characterization of bacteriocins from *Lactococcus lactis, Enterococcus faecium* and *Enterococcus mundtii* strains isolated from turbot (*Psetta maxima*). Food Res. Int. **39:**356-364.
4. **de Kwaadsteniet, M., T. Fraser, C. A. van Reenen, and L. M. T. Dicks.** 2006. Bacteriocin T8, a novel class IIa *sec*-dependent bacteriocin produced by *Enterococcus faecium* T8, isolated from the vaginal secretions of children infected with the human immunodeficiency virus. Appl. Environ. Microbiol. **72:**4761-4766.
5. **Dellaglio, F. V., V. Bottazzi, and L. D. Trovatelli.** 1973. Deoxyribonucleic acid homology and base composition in some thermophilic lactobacilli. J. Gen. Microbiol. **74:**289-297.
6. **de Vuyst, L., and E. J. Vandamme (ed).** 1994. Bacteriocins of lactic acid bacteria: microbiology, genetics and application. Blackie Academic & Professional, Chapman & Hall. LTD. London.
7. **Engelke, G., Z. Gutowski-Eckel, M. Hammelmann, and K. D. Entian.** 1992. Biosynthesis of the lantibiotic nisin: genomic organization and membrane localization of the NisB protein. Appl. Environ. Microbiol. **58:**3730-3743.
8. **Engelke, G., Z. Gutowski-Eckel, P. Kiesau, K. Siegers, M. Hammelmann, and K. D. Entian.** 1994. Regulation of nisin biosynthesis and immunity in *Lactococcus lactis* 6F3. Appl. Environ. Microbiol. **60:**814-825.
9. **Felske, A., H. Rheims, A. Wolterink, E. Stackebrandt, and A. D. L. Akkermans.** 1997. Ribosome analysis reveals prominent activity of an uncultured member of the class Actinobacteria in grassland soils. Microbiology **143:**2983-2989.
10. **Gross, E., and J. L. Morell.** 1971. The structure of nisin. J. Am. Chem. Soc. **93:**4634-4635.

11. **Holt, J. G (ed).** 1994. Bergey's manual of determinative bacteriology. Williams & Wilkins, Baltimore, MD.
12. **Ivanova, I., V. Miteva, T. Stefanova, A. Pantev, I. Budakov, S. Danova, P. Moncheva, I. Nikolova, X. Dousset, and P. Boyaval.** 1998. Characterization of a bacteriocin produced by *Streptococcus thermophilus* 81. Int. J. Food Microbiol. **42:**147-158.
13. **Kruszewska, D., H. G. Sahl, G. Bierbaum, U. Pag, S. O. Hynes, and A. Ljungh.** 2004. Mersacidin eradicates methicillin-resistant *Staphylococcus aureus* (MRSA) in a mouse rhinitis model. J. Antimicrob. Chemother. **54:**648-653.
14. **Mattick, A. T. R., and A. Hirsch.** 1947. Further observation on an inhibitory substance (nisin) from lactic streptococci. Lancet **ii:**5-12.
15. **Mulders, J. W., I. J. Boerrigter, H. S. Rollema, R. J. Siezen, and W. M. de Vos.** 1991. Identification and characterization of the lantibiotic nisin Z, a natural nisin variant. Eur. J. Biochem. **201:**581-584.
16. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2 nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Schägger, H., and G. von Jagow.** 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal. Biochem. **166:**368-379.
18. **Stein, T., S. Heinzmann, I. Solovieva, and K. D. Entian.** 2003. Function of *Lactococcus lactis* nisin immunity genes *nisI* and *nisFEG* after coordinated expression in the surrogate host *Bacillus subtilis*. J. Biol. Chem. **278:**89-94.
19. **Todorov, S. D., and L. M. T. Dicks.** 2005. Characterization of bacteriocins produced by lactic acid bacteria isolated from spoiled black olives. J. Basic Microbiol. **45:**312-322.
20. **van der Meer, J. R., J. Polman, M. M. Beerthuyzen, R. J. Siezen, O. P. Kuipers, and W. M. de Vos.** 1993. Characterization of the *Lactococcus lactis* nisin A operon genes *nisP*, encoding a subtilisin-like serine protease involved in precursor processing, and *nisR*, encoding a regulatory protein involved in nisin biosynthesis. J. Bacteriol. **175:**2578-2588.
21. **van Reenen, C. A., L. M. T. Dicks, and M. L. Chikindas.** 1998. Isolation, purification and partial characterization of plantaricin 423, a bacteriocin produced by *Lactobacillus plantarum.* J. Appl. Microbiol. **84:**1131-1137.

22. **Zendo, T., M. Fukao, K. Ueda, T. Higuchi, J. Nakayama, and K. Sonomoto.** 2003. Identification of the lantibiotic nisin Q, a new natural nisin variant produced by *Lactococcus lactis* 61-14 isolated from a river in Japan. Biosci. Biotechnol. Biochem. **67:**1616-1619.

TABLE 1. Target strains, growth conditions and spectrum of antimicrobial activity

Target strain(s)[a]	Growth medium and conditions	Bacteriocin activity
Lactobacillus acidophilus LMG 13550	MRS, 37°C anaerobic	-
Lactobacillus bulgaricus LMG 13551	MRS, 37°C anaerobic	-
Lactobacillus casei LMG 13552	MRS, 37°C anaerobic	-
Lactobacillus curvatus LMG 13553	MRS, 30°C anaerobic	++
Lactobacillus fermentum LMG 13554	MRS, 37°C anaerobic	-
Lactobacillus helveticus LMG 13555	MRS, 42°C anaerobic	-
Lactobacillus plantarum LMG 13556	MRS, 37°C anaerobic	++
Lactobacillus reuteri LMG 13557	MRS, 37°C anaerobic	+
Lactobacillus sake LMG 13558	MRS, 30°C anaerobic	-
Pediococcus pentosaceus LMG 13560, LMG 13561	MRS, 30°C anaerobic	-
Leuconostoc cremoris LMG 13562, LMG 13563	MRS, 30°C anaerobic	-
Streptococcus thermophilus LMG 13564, LMG 13565	MRS, 42°C anaerobic	-
Enterococcus faecalis LMG 13566	BHI, 37°C anaerobic	-
Staphylococcus carnosus LMG 13567	BHI, 37°C anaerobic	+++
Listeria innocua LMG 13568	BHI, 30°C anaerobic	-
Bacillus cereus LMG 13569	BHI, 37°C anaerobic	-
Clostridium sporogenes LMG 13570	RCM[b], 37°C anaerobic	-
Clostridium tyrobutyricum LMG 13571	RCM, 30°C anaerobic	-
Propionibacterium sp. LMG 13574	GYP[c], 32°C anaerobic	-
Staphylococcus aureus K (own collection)	BHI, 37°C aerobic	+++
Staphylococcus aureus J (own collection)	BHI, 37°C aerobic	+++

[a] LMG, strains received from the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium, now deposited into the Belgium Co-ordinated Collection of Micro-organisms(BCCM)/LMG Culture Collection(http://bccm.belspo.be/about/lmg.php).

[b]RCM, reinforced clostridium medium

[c]GYP, glucose-yeast-peptone medium

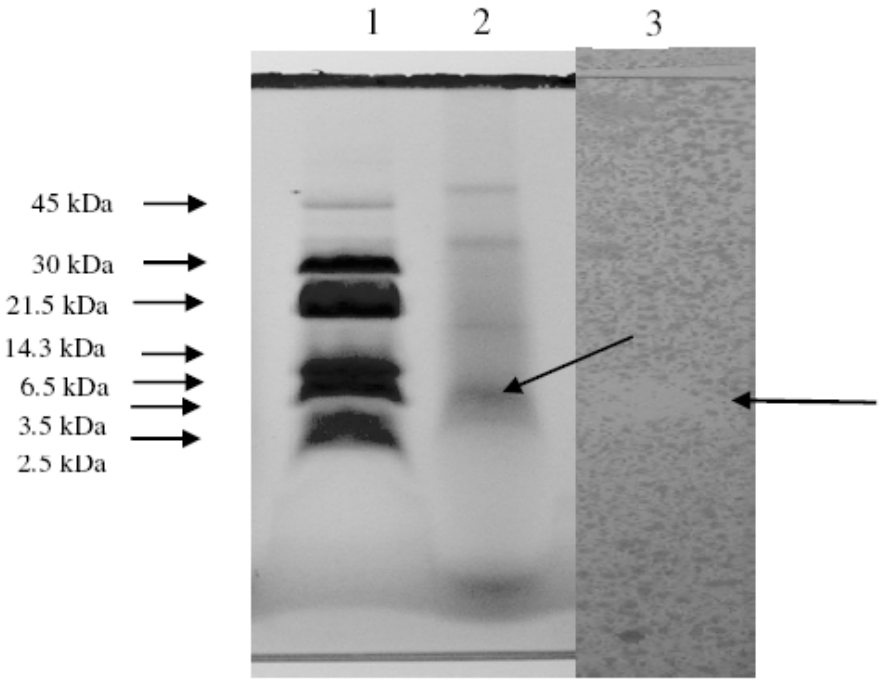

FIG. 1. Separation of partially purified nisin F by SDS-PAGE. Lane 1: Rainbow low-range protein size marker RPN755 (Amersham International, UK). Lane 2: Protein band representing nisin F (arrow). Lane 3: Inhibition of *S. aureus* K (arrow), embedded in BHI soft agar (1%).

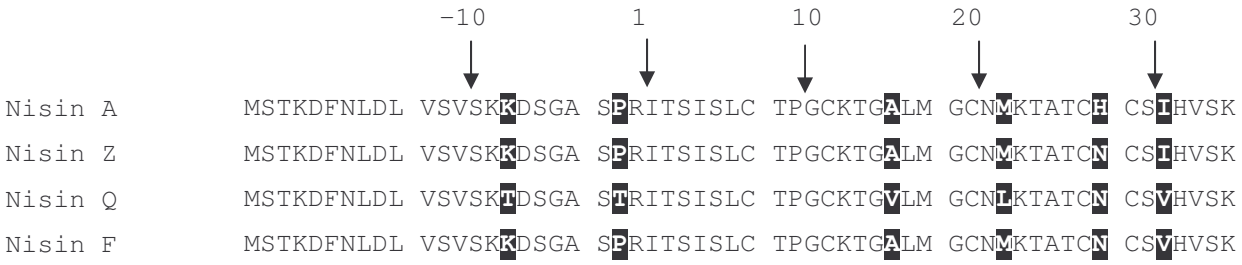

FIG. 2. Amino acid sequences of nisin F, deduced from the DNA sequence (GenBank accession number EU057979), and compared to the amino acid sequences of nisin A, nisin Z and nisin Q. The leader peptide of each nisin variant consists of 13 amino acids (from position -13 to -1), followed by amino acids encoding the mature protein (amino acid positions 1 to 34). Differences in amino acids are indicated with a black background.

Chapter 4

Nisin F in the treatment of respiratory tract infections caused by *Staphylococcus aureus*

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Aims: To determine the antimicrobial activity of nisin F against *Staphylococcus aureus* in the respiratory tract.

Methods and Results: The respiratory tract of non-immunosuppressed and immunosuppressed Wistar rats were colonised with 4 x 10^5 viable cells of *S. aureus* K and then treated by administering 8192 arbitrary units (AU) nisin F intranasal. Symptoms of pneumonia were detected in the trachea and lungs of immunosuppressed rats that had not been treated with nisin F. The trachea and lungs of immunosuppressed rats treated with nisin F were healthy. No significant differences were recorded in blood cell indices. The antimicrobial activity of low concentrations nisin F (80 - 320 AU ml^{-1}) was slightly stimulated by lysozyme and lactoferrin.

Conclusions: Nisin F inhibited the growth of *S. aureus* K in the respiratory tract of immunocompromised rats. Treatment with nisin F at 8192 AU proofed safe, as the trachea, lungs, bronchi and haematology of the rats appeared normal.

Significance and Impact of the Study: Nisin F is nontoxic and may be used to control respiratory tract infections caused by *S. aureus*. This is, however, a preliminary study with an animal model and need to be confirmed with studies on humans.

Keywords

nisin F, *Staphylococcus aureus*, respiratory tract

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Introduction

Staphylococcus aureus is an important pathogen in upper (Brook 2005; Grzegorowski and Szydlowski 2005; Verhoeff *et al*. 2006) and lower respiratory tract infections (Harmanci *et al.* 2002; Saiman 2004; Weber *et al*. 2007). Development of new antistaphylococcal agents is driven by the emergence of resistance to methicillin, macrolides, lincosamides, flouroquinlones, tetracyclines, aminoglycosides, chloramphenicol (Almer *et al.* 2002), vancomycin (Centers for Disease Control and Prevention 2004), mupirocin (Watanabe *et al.* 2001) and the more recently developed antimicrobials quinupristin-dalfopristin (Fagon *et al.* 2000), linezolid (Peeters and Sarria 2005) and daptomycin (Skiest 2006).

Lysozyme and lactoferrin are the two most abundantly secreted antimicrobial proteins in airway secretions of humans (Brogan *et al*. 1975; Harbitz *et al*. 1984; Wilson 2005). These proteins are antimicrobial against *S. aureus* and have a synergistic effect with nisin (Nattress *et al.* 2001; Murdock *et al.* 2007). The protective effect of lactic acid bacteria against respiratory tract infections has been studied in animal models (Alvarez *et al*. 2001; Racedo *et al*. 2006) and humans (Glück and Gebbers 2003; Cobo Sanz *et al*. 2006). Studies on the protective effect of bacteriocins produced by lactic acid bacteria against respiratory tract infections are limited. Kruszewska *et al*. (2004) repressed the growth of methicillin-resistant *S. aureus* (MRSA) strains in the respiratory tract of mice with mersacidin, a lantibiotic produced by *Bacillus* sp. HIL Y-85 54728. Similar studies with nisin, a lantibiotic produced by *Lactococcus lactis* subsp. *lactis*, did not eradicate *S. aureus* from the nasal tract of rats (Kokai-Kun *et al.* 2003).

In a previous paper (De Kwaadsteniet *et al*. 2008), we described nisin F produced by *L. lactis* subsp. *lactis* F10 and reported on the *in vitro* activity against clinical strains of *S. aureus.* In the present study, non-immunosuppressed and immunosuppressed rats were intranasally infected with a clinical strain of *S. aureus* and then treated with nisin F. Blood cell counts and the histology of lung and trachea tissue from rats in both groups were compared. The safety of nisin F was determined by administering the peptide to non-immunosuppressed and immunosuppressed rats intranasally.

Materials and methods

Bacterial strains and culture conditions

Lactococcus lactis subsp. *lactis* F10 was cultured in De Man Rogosa and Sharpe (MRS) broth (Biolab, Biolab Diagnostics, Midrand, South Africa) at 30°C and *Staphylococcus aureus* K in brain heart infusion (BHI, Biolab) at 37°C. Biard-Parker agar base (Biolab) was used as selective medium for *S. aureus* K, isolated from the nasal cavity of a patient with acute sinusitis.

Nisin F preparation

Nisin F, produced by *L. lactis* subsp. *lactis* F10 (De Kwaadsteniet *et al.* 2008), was semi-purified by ammonium sulphate precipitation and dialysed according to the method described by Sambrook *et al*. (1989). The peptide was concentrated by freeze-drying and resuspended in sterile physiological saline. Antimicrobial activity was determined by using the agar-spot test method and expressed as arbitrary units (AU) per millilitre. One AU is the reciprocal of the highest serial two-fold dilution showing a clear zone of inhibition of the indicator strain (Van Reenen *et al*. 1998). The indicator strain was an 18-h-old culture of *S. aureus* K (10^6 CFU ml^{-1}), embedded in BHI soft agar (1%).

Synergistic effect of nisin F with lysozyme and lactoferrin

The antimicrobial activity of nisin F, lysozyme, lactoferrin and combinations thereof were tested as follows: Nisin F was added to 100 µl BHI to yield final concentrations of 1280, 320, and 160AU ml^{-1}, respectively (Table 1). In another set, lysozyme (Roche Diagnostic Gmbh, Manheim, Germany) and lactoferrin (Sigma Diagnostics, St. Louis, USA) were added to 100 µl BHI to yield a final concentration of 500 µg ml^{-1} (Table 1). Combinations of nisin F, lysozyme and lactoferrin were also prepared (Table 1). The suspensions were inoculated into separate wells of a sterile microtitre plate and then inoculated with *S. aureus* K to yield 10^5 viable cells. Nisin F, lysozyme and lactoferrin at the same concentrations, but not inoculated with *S. aureus* K served as controls. The microtitre plate was incubated at 37ºC for 24 h and OD-readings taken at 595 nm (Model 680 Microplate Reader, Hercules, CA, USA, Bio-Rad). All experiments were carried out in triplicate.

Animal model and inoculation procedure

Approval for the experiments was obtained from the Animal Ethics Committee of Stellenbosch University (ethics reference number 2005B02003). Wistar male rats were divided into eight groups and housed in plastic cages in animal rooms with constant environmental conditions. Each group contained six rats. All rats received a standard rodent diet. The immunity of rats in groups 3 and 4 was suppressed by adding dexamethasone (2.5 mg l^{-1}) to their drinking water for the first six days. An inoculum of *S. aureus* K was prepared by growing the cells in BHI for 18 h at 37°C. The cells were harvested (10 000 g, 10 min, 4°C), washed three times with 10 ml sterile physiological saline, resuspended in 10 ml sterile skim milk (10% w/v) and 4 ml dispensed into freeze-drying ampules. After 24 h of freeze-drying, the cells were resuspended in sterile saline to 10^7 CFU ml^{-1}. On days 5, 6, 7, and 8 the rats in the groups 1 to 4 were intranasally infected with 4 x 10^5 viable cells of *S. aureus* K (2 x 10 µl of 10^7 CFU ml^{-1} per nostril) per day. On days 9, 10, 11 and 12 rats in groups 1 and 3 were treated with 8192 AU nisin F (2 x 10 µl per nostril) and rats in groups 2 and 4 with sterile physiological saline (2 x 10 µl per nostril).

The safety of nisin F was tested by conducting a similar experiment. Rats in groups 7 and 8 received dexamethasone for the first six days as described before. On days 9, 10, 11 and 12 rats in groups 5 and 7 received 8912 AU nisin F and groups 6 and 8 sterile physiological saline, as described before. None of the rats in groups 5 to 8 were infected with *S. aureus* K.

On day 13 all the rats were euthanized by an overdose with pentobarbitone sodium (Centaur Labs, Bayer Animal Health Isando, South Africa) administered intraperitoneally.

Testing for adverse effects

The rats were weighed and their feed and water intake determined on a daily basis. Blood from rats infected with *S. aureus* K was plated onto Baird-Parker agar (Biolab) and the plates incubated at 37°C for 24 h.

Histological studies

The trachea, bronchi and lungs of three rats from each group were aseptically removed, fixed in 4% (v/v) formaldehyde (PBS) for 24 h at 25ºC, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). The samples were processed and analysed at Pathcare

Veterinary Pathologists (Pathcare, Dietrich, Voigt, Mia and Partners, Goodwood, South Africa).

Haematology

Blood samples were collected from the rats directly after they were euthanized. Total blood counts were determined on fresh blood with an automated haematology counter (Beckman Coulter Hematology Analyzer LH; Beckman Coulter, Inc., CA, USA). All tests were conducted by Pathcare Veterinary Pathologists.

Results

Nisin preparation and dosage

The antimicrobial activity of concentrated semi-purified nisin F was 204 800 AU ml^{-1}, as determined by using *S. aureus* K as target strain. The daily dosage with nisin F was thus 8192 AU (2 x 10 µl per nostril) per rat for both the infection and nisin F toxicity studies.

Effect of nisin F, alone and in combination with lysozyme and lactoferrin, on the growth of *S. aureus* K

Changes recorded in OD-readings of *S. aureus* K after incubation in the presence of nisin F, nisin F supplemented with lysozyme and lactoferrin, and lysozyme and lactoferrin without nisin F, are listed in Table 1. In the absence of nisin F, the growth of *S. aureus* K increased to OD (595 nm) = 2.0. Nisin at 1280 AU ml^{-1} prevented the growth of *S. aureus* K (OD_{595nm} = 0.1). However, low concentrations of nisin F (160 to 320 AU ml^{-1}) stimulated the growth of *S. aureus* K slightly (OD_{595nm} = 0.9 to 1.1). No significant differences in the growth of *S. aureus* K were recorded when the cells were treated with nisin F (1280 AU ml^{-1}) and the same level of nisin F in combination with either lysozyme or lactoferrin (OD_{595nm} = 0.1 to 0.2). Nisin F at 320 AU ml^{-1} combined with lysozyme completely inhibited the growth of *S. aureus* K (OD_{595nm} = 0). Synergism was observed between nisin F (at 320 and 160 AU ml^{-1}) in combination with either lysozyme and lactoferrin.

Testing for adverse effects

No significant differences were recorded in weight gain, and water and feed intake for rats in groups 1 – 4 (not shown). Bacterial growth was recorded in blood sampled from one of the rats in group 4 (immunosuppressed, not treated with nisin F and infected with *S. aureus* K). Small (1-5 mm) black colonies, surrounded by clear zones, characteristic of *S. aureus*, were visible on the Baird-Parker agar plates. The lungs of the same infected animal had clearly visible necrotic lesions (not shown). No bacteremia or necrotic lesions were observed on lungs of rats in groups 1, 2 and 3. No significant differences were recorded in weight gain, and water and feed intake for rats in the nisin toxicity trial (groups 5 - 8).

Histology

No significant histopathology were recorded in lung tissue collected from rats in group 1 (non-immunosuppressed, infected with *S. aureus* K and treated with nisin F), group 2 (non immunosuppressed, infected with *S. aureus* K and treated with sterile physiological saline), and group 3 (immunosuppressed, infected with *S. aureus* K, and treated with nisin F) (Fig. 1a). Severe symptoms of pneumonia were detected in lung tissue sampled from rats in group 4 (immunosuppressed, infected with *S. aureus* K, and treated with sterile physiological saline). The alveoli of the lungs from rats in group 4 were clearly obliterated due to the infiltration of macrophages, granulocytes and lymphocytes, and the proliferation of fibroblasts and alveolar epithelial cells (Fig. 1b). The trachea of the same rats showed signs of mucosal hyperplasia, with infiltration of lymphocytes and a few mast cells (Fig. 1d). No mucosal hyperplasia was observed in the trachea of rats from group 3 (immunosuppressed, infected with *S. aureus* K, and treated with nisin F) (Fig. 1c). No histological changes or differences were detected in the bronchi of rats in groups 1 to 4.

The histology of the trachea, lungs and bronchi of non-immunosuppressed and immunosuppressed rats that have been treated with nisin F (groups 5 and 7) and sterile physiological saline (groups 6 and 8), and not infected with *S. aureus* K, appeared healthy (not shown).

Haematology

The number of white blood cells, lymphocytes and neutrophils recorded in blood samples collected from non-immunosuppressed and immunosuppressed rats that have been infected with *S. aureus* K (groups 1 - 4) did not differ significantly (Fig. 2). The number of white blood cells, lymphocytes and neutrophils of the rats in the nisin F toxicity trial (Fig. 2) also did not differ between the nisin-treated rats (groups 5 and 7) and saline-treated rats (groups 6 and 8). No significant differences were detected in the red blood cell indices (Table 2) between the experimental and control groups for both the infection trial, i.e. groups 1 and 2 for non-immunosuppressed rats and groups 3 and 4 for immunosuppressed rats, and for the nisin F toxicity trial, i.e. groups 5 and 6 for non-immunosuppressed rats and groups 7 and 8 for immunosuppressed rats (Table 2). The red blood cell distribution width (RDW) of rats that have not been infected with *S. aureus* K (groups 5 - 8) was slightly higher compared to rats that have been infected (groups 1 - 4).

Discussion

Growth of *S. aureus* K was clearly inhibited by high levels of nisin (1280 AU ml^{-1}), whether tested alone, or in combination with lysozyme and lactoferrin. Less growth inhibition of *S. aureus* K at lower concentrations of nisin F (160 - 320 AU ml^{-1}) was to be expected. It is also not surprising to have recorded a slight increase in antimicrobial activity when nisin F was tested in combination with lysozyme and lactoferrin. This is indicative of synergistic antimicrobial activity, perhaps easier to observe in the presence of lower levels of nisin (160 - 320 AU ml^{-1}) than an overwhelming concentration (1280 AU ml^{-1}). Complete growth inhibition of *S. aureus* K was observed when 320 AU ml^{-1} nisin F was tested in combination with lysozyme.

Lysozyme and lactoferrin are present at relatively high concentrations in the upper respiratory tract of humans and in sputum at approximately 500 μg ml^{-1} (Brogan *et al.* 1975; Harbitz *et al*. 1984; Wilson 2005). Previous studies have shown a synergistic effect between nisin and lysozyme (Nattress *et al*. 2001) and nisin and lactoferrin (Murdock *et al*. 2007). Nisin prevents cell growth by binding to the cell wall precursor lipid II and destabilising the cell membrane, whereas lysozyme damages the glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid residues in the peptidoglycan (Ganz 2004).

Lactoferrin inhibits microbial growth by sequestering iron involved in respiration (Arnold *et al.* 1977).

Non-immunosuppressed rats dosed with *S. aureus* K did not develop symptoms of respiratory tract infections, confirming previous reports that the immune system of rats has to be compromised when studying *S. aureus* respiratory tract infections (Kruszewska *et al.* 2004). *S. aureus* have been associated with pneumonia, especially nosocomial pneumonia (Weber *et al*. 2007) and community-acquired pneumonia (Micek *et al*. 2007). It is thus not surprising that immunosuppressed rats infected with *S. aureus* K and not treated with nisin F developed severe symptoms of pneumonia, i.e. obliterated alveoli in lungs and mucosal hyperplasia in trachea. Mucosal hyperplasia may be due to increased production of mucus in the infected area (Fig. 1d). This is expected, as the up-regulation of mucin genes from by products produced by *S. aureus* has been reported (Dohrman *et al.* 1998; Basbaum *et al*. 1999).

Necrotic lesions observed in the lung tissue and bacteremia detected in immunosuppressed rats infected with *S. aureus* K is indicative of damaged lung tissue through which the pathogen migrates into the blood stream. Immunosuppressed and infected rats treated with nisin F had no symptoms of pneumonia, mucosal hyperplasia, blood contamination or necrotic lesions, suggesting that the lantibiotic suppressed the growth of *S. aureus* K *in vivo*. Kokai-Kun *et al*. (2003), on the other hand, reported no inhibition of *S. aureus* in the nasal tract of cotton rats and concluded that nisin is either inactivated or absorbed in the nares. Another possible explanation is the formation of biofilms in the nasal tract, which could render the strains more resistant to nisin (Bendouah *et al*. 2006).

No histology changes were observed in the lungs, trachea and bronchi of rats in the nisin F toxicity trial (non-immunosuppressed and immunosuppressed groups). No differences in weight gain and food and water consumption were recorded. Nisin F had no effect on the white cell, lymphocyte and neutrophil production in the rat model. The red blood cell indices of rats treated with nisin F was similar to that recorded for rats treated with saline. Based on these results, nisin F is nontoxic and safe to use in the treatment of upper-respiratory infections.

As far as we could determine, this is the first successful report on a nisin variant being used to control intranasal *S. aureus* infections. Although murine models serve as an essential link between *in vitro* models and human clinical trails, the data presented in this study is preliminary and will have to be confirmed with studies on human volunteers before nisin F may be declared safe for human application.

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References

Almer, L.S., Shortridge, V.D., Nilius, A.M., Beyer, J.M., Soni, N.B., Bui, M.H., Stone, G.G. and Flamm, R.K. (2002) Antimicrobial susceptibility and molecular characterization of community-acquired methicillin-resistant *Staphylococcus aureus*. *Diagn Microbiol Infect Dis* **43,** 225-232.

Alvarez, S., Herrero, C., Bru, E. and Perdigon, G. (2001) Effect of *Lactobacillus casei* and yogurt administration on prevention of *Pseudomonas aeruginosa* infection in young mice. *J Food Prot* **64,** 1768-1774.

Arnold, R.R., Cole, M.F. and McGhee, J.R. (1977) A bactericidal effect of human lactoferrin. *Science* **15,** 263-265.

Basbaum, C., Lemjabbar, H., Longphre, M., Li, D., Gensch, E. and McNamara, N. (1999) Control of mucin transcription by diverse injury-induced signaling pathways. *Am J Respir Crit Care Med* **160,** S44-S48.

Bendouah, Z., Barbeau, J., Hamad, W.A. and Desrosiers, M. (2006) Biofilm formation by *Staphylococcus aureus* and *Pseudomonas aeruginosa* is associated with an unfavorable evolution after surgery for chronic sinusitis and nasal polyposis. *Otolaryngol Head Neck Surg* **134,** 991-996.

Brogan, T. D., Ryley, H. C., Neale, L. and Yassa, J. (1975) Soluble proteins of bronchopulmonary secretions from patients with cystic fibrosis, asthma, and bronchitis. *Thorax* **30,** 72-79.

Brook, I. (2005) Microbiology and antimicrobial management of sinusitis. *J Laryngol Otol* **119,** 251-258.

Centers for Disease Control and Prevention (CDC). (2004) Vancomycin-resistant *Staphylococcus aureus*--New York, 2004. *MMWR Morb Mortal Wkl Rep* **53,** 322-323.

Cobo Sanz, J.M., Mateos, J.A. and Munoz Conejo, A. (2006) Effect of *Lactobacillus casei* on the incidence of infectious conditions in children. *Nutr Hosp* **21,** 547-551.

De Kwaadsteniet, M., Ten Doeschate, K. and Dicks, L.M.T. (2008) Characterization of the structural gene encoding nisin F, a new lantibiotic produced by *Lactococcus lactis* subsp. *lactis* isolate from freshwater fish (*Clarius gariepinus*). *Appl Environ Microbiol* **74,** 547-549.

Dohrman, A., Miyata, S., Gallup, M., Li, J.D., Chapelin, C., Coste, A., Escudier, E., Nadel, J. *et al.* (1998) Mucin gene (*MUC 2* and *MUC 5AC*) upregulation by Gram-positive and Gram-negative bacteria. *Biochim Biophys Acta* **1406,** 251-259.

Fagon, J., Patrick, H., Haas, D.W., Torres, A., Gilbert, C., Cheadle, W.G., Falcone, R.E., Anholm, J.D. *et al* (2000) Treatment of Gram-positive nosocomial pneumonia. Prospective randomized comparison of quinupriston/dalfopristin versus vancomycin. Nosocomial Pneunomia Group. *Am J Respir Crit Care Med* **161,** 753-762.

Ganz, T. (2004) Antimicrobial polypeptides. *J Leukoc Biol* **75,** 34-38.

Glück, U. and Gebbers, J.O. (2003) Ingested probiotics reduce nasal colonization with pathogenic bacteria (*Staphylococcus aureus, Streptococcus pneumoniae*, and hemolytic streptococci). *Am J Clin Nutr* **77,** 517-520.

Grzegorowski, M. and Szydlowski, J. (2005) Acute otitis media in children. *Pol Merkur Lekarski* **19,** 494-496.

Harbitz, O., Jenssen, A. O. and Smidsrød, O. (1984) Lysozyme and lactoferrin in sputum from patients with chronic obstructive lung disease. *Eur J Respir Dis* **65,** 512-520.

Harmanci, A., Harmanci, O. and Akova, M. (2002) Hospital-acquired pneumonia: challenges and options for diagnosis and treatment. *J Hosp Infect* **51,** 160-167.

Kokai-Kun, J.F., Walsh, S.M., Chanturiya, T. and Mond, J.J. (2003) Lysostaphin cream eradicates *Staphylococcus aureus* nasal colonization in a cotton rat model. *Antimicrob Agents Chemother* **47,** 1589-1597.

Kruszewska, D., Sahl, H.G., Bierbaum, G., Pag, U., Hynes, S.O. and Ljungh, A. (2004) Mersacidin eradicates methicillin-resistant *Staphylococcus aureus* (MRSA) in a mouse rhinitis model. *J Antimicrob Chemother* **54,** 648-653.

Micek, S.T., Kollef, K.E., Reichley, R.M., Roubinian, N. and Kollef, M.H. (2007) Healthcare-associated pneumonia and community-acquired pneumonia: a single-center experience. *Antimicrob Agents Chemother* **51,** 3568-3573.

Murdock, C.A., Cleveland, J., Matthews, K.R. and Chikindas, M.L. (2007) The synergistic effect of nisin and lactoferrin on the inhibition on *Listeria monocytogenes* and *Escherichia coli* O157:H7. *Lett Appl Microbiol* **44,** 255-261.

Nattress, F.M., Yost, C.K. and Baker, L.P. (2001) Evaluation of the ability of lysozyme and

nisin to control meat spoilage bacteria. *Int J Food Microbiol* **70,** 111-119.

Peeters, M.J. and Sarria, J.C. (2005) Clinical characteristics of linezolid-resistant *Staphylococcus aureus* infections. *Am J Med Sci* **330,** 102-104.

Racedo, S., Villena, J., Medina, M., Aguero, G., Rodriquez, V. and Alvarez, S. (2006) *Lactobacillus casei* administration reduces lung injuries in a *Streptococcus pneumoniae* infection in mice. *Microbes Infect* **8,** 2359-2366.

Saiman, L. (2004) Microbiology of early CF lung disease. *Paediatr Respir Rev* **5,** S367-S369.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular cloning: A Laboratory Manual,* 2 nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Skiest, D.J. (2006) Treatment failure resulting from resistance of *Staphylococcus aureus* to daptomycin. *J Clin Microbiol* **44,** 655-656.

Van Reenen, C.A., Dicks, L.M.T. and Chikindas, M.L. (1998) Isolation, purification and partial characterization of plantaricin 423, a bacteriocin produced by *Lactobacillus plantarum. J Appl Microbiol* **84,** 1131-1137.

Verhoeff, M., van der Veen, E.L., Rovers, M.M., Sanders, E.A.M. and Schilder, A.G.M. (2006) Chronic suppurative otitis media: A review. *Int J Pediatr Otorhinolaryngol* **70,** 1-12.

Watanabe, H., Masaki, H., Asoh, N., Watanabe, K., Oishi, K., Furumoto, A., Kobayashi, S., Sato, A. and Nagatake, T. (2001) Emergence and spread of low-level mupirocin resistance in methicillin-resistant *Staphylococcus aureus* isolated from a community hospital in Japan. *J Hosp Infect* **47,** 294-300.

Weber, D.J., Rutala, W.A., Sickbert-Bennett, E.E., Samsa, G.P., Brown, V. and Niederman, M.S. (2007) Microbiology of ventilator-associated pneumonia compared with that of hospital-acquired pneumonia. *Infect Control Hosp Epidemiol* **28,** 825-831.

Wilson, M. (2005) Microbial Inhabitants of Humans. Their Ecology and Role in Health. Cambridge, UK: Cambridge University Press.

Table 1 Optical density readings (at 595 nm) of *S. aureus* K after incubation with nisin F, lysozyme, lactoferrin, and a combination of nisin F and lysozyme or lactoferrin, respectively

	In the absence of lysozyme and lactoferrin	Lysozyme 500 μg ml^{-1}	Lactoferrin 500 μg ml^{-1}
Nisin F 1280 AU[a] ml^{-1}	0.08±0.06	0.14±0.02	0.15±0.04
Nisin F 320 AU ml^{-1}	1.11±0.23	0	0.43±0.11
Nisin F 160 AU ml^{-1}	0.89±0.06	0.44±0.01	0.47±0.02
		1.66±0.04	0.88±0.03

Standard deviation displayed is a mean value of three repeats per experiment (n = 3).

AU, antimicrobial units.

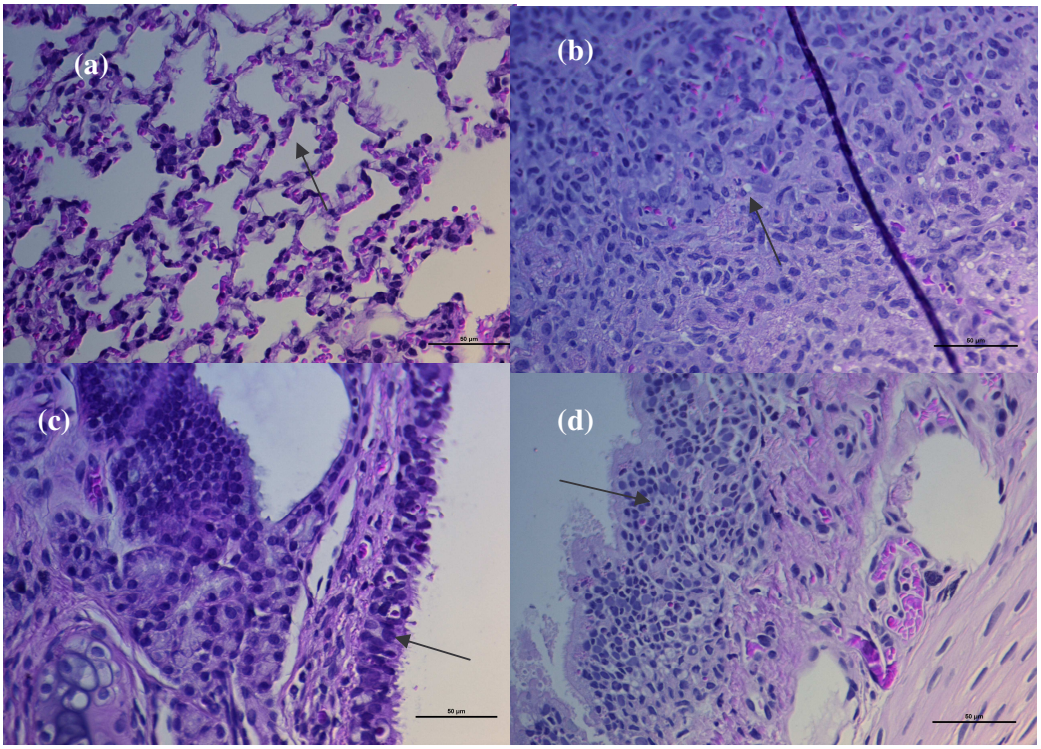

Figure 1 Histological images of respiratory tract tissue from immunosuppressed rats infected with *S. aureus* K and then treated with either nisin F or sterile physiological saline. (a, c) Lungs and trachea, respectively, of rats from group 3 (immunosuppressed, infected and treated with nisin F). (b, d) Lungs and trachea, respectively, of rats from group 4 (immunosuppressed, infected and treated with saline). Differences observed in alveoli and trachea (no mucosal hyperplasia compared to mucosal hyperplasia) is shown by arrows. Magnification: 400x

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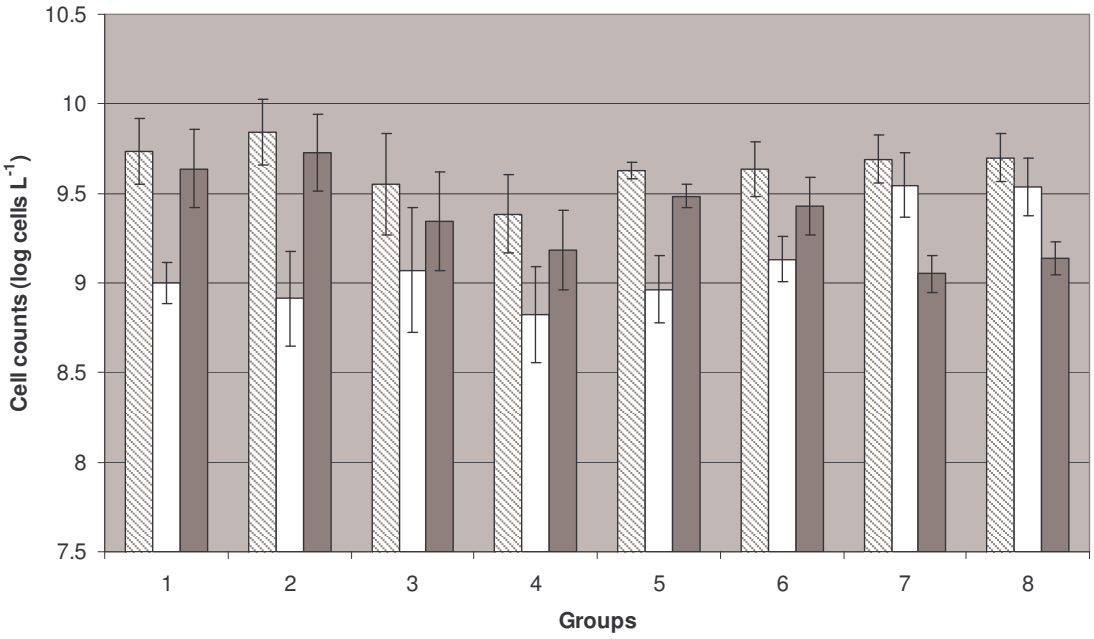

Figure 2 Cell counts of total white blood cells (striped column), neutrophils (white column), and lymphocytes (grey column). Group 1: infected, non-immunosuppressed nisin F-treated rats, group 2: infected, non-immunosuppressed saline treated, group 3: infected, immunosuppressed nisin F-treated rats, group 4: infected, immunosuppressed saline treated rats, group 5: noninfected non-immunosuppressed nisin F-treated rats, group 6: noninfected non-immunosuppressed saline treated group, group 7: noninfected immunosuppressed nisin F-treated rats and group 8: noninfected immunosuppressed saline treated rats.

Table 2 The red blood cell indices

Red blood cell indices	Group[e] 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Haemoglobin (g dL^{-1})	14.85±0.78	14.92±0.92	15.17±0.72	15.73±0.54	14.1±0.16	13.70±0.37	13.08±0.61	12.45±1.21
Haematocrit (L^{-1})	0.42±0.04	0.43±0.03	0.44±0.02	0.46±0.01	0.41±0.01	0.38±0.02	0.38±0.02	0.37±0.03
MCV[a] (femtoliters)	59.7±0.5	58.5±1.1	58.8±1.2	57.2±1.8	58.5±1.9	59.83±2.0	63.83±1.9	63±3.4
MCH[b] (pg)	21.0±0.9	20.5±0.6	20.3±0.5	19.5±0.6	20.25±0.5	21.17±1.2	21.67±0.8	21.33±0.8
MCHC[c] (g dL^{-1})	35.3±1.2	35.3±0.8	34.3±0.5	34.3±0.5	34.75±0.5	35.67±0.8	34.33±0.5	33.83±1.2
RDW[d] (%)	11.4±0.5	11.1±0.2	12.4±0.5	11.6±0.3	14.48±0.8	15.27±0.9	17.27±1.2	18.12±3.7

[a]MCV, mean corpuscular volume

[b]MCH, mean corpuscular haemoglobin

[c]MCHC, mean corpuscular haemoglobin concentration

[d]RDW, red blood cell distribution width

[e]Group 1: infected, non-immunosuppressed nisin F-treated rats, group 2: infected, non-immunosuppressed saline treated, group 3: infected, immunosuppressed nisin F-treated rats, group 4: infected, immunosuppressed saline treated rats, group 5: noninfected non-immunosuppressed nisin F-treated rats, group 6: noninfected non-immunosuppressed saline treated group, group 7: noninfected immunosuppressed nisin F-treated rats and group 8: noninfected immunosuppressed saline treated rats

Chapter 5

Nisin F in the treatment of subcutaneous skin infections by monitoring bioluminescent *Staphylococcus aureus* noninvasively and in real-time

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The potential of nisin F as an antimicrobial agent in treating subcutaneous skin infections was tested *in vivo* by infecting C57BL/6 mice with a bioluminescent *Staphylococcus aureus* strains (Xen 36). Strain Xen 36 has the *luxABCDE* operon located on a native plasmid. Mice were grouped into four groups. Infected with strain Xen 36 and treated with nisin F, infected with strain Xen 36 and treated with saline (placebo), not infected and treated with nisin (control) and not infected and not treated (control). The immune systems of the mice were suppressed with deksamethasone. Mice were treated with either nisin F or sterile physiological saline 24 h and 48 h after infection with subcutaneously injected *S. aureus* Xen 36 (4 x 10^6 cfu). Histology and bioluminescent flux measurements revealed no significant difference between infected mice treated with nisin and saline, respectively. However, infected mice treated with nisin F had an increased number of polymorphonuclear cells when compared with infected mice treated with saline. Also, not infected mice treated with nisin F had an influx of polymorphonuclear cells. Nisin F is ineffective in combating deep dermal staphylococcal infections. The apparent immune modulation that subcutaneously infected nisin had needs to be further investigated.

Keywords

nisin F, *Staphylococcus aureus*, subcutaneous skin infection

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Introduction

Skin and soft tissue infections (SSTIs) are an immense health problem with infections ranging from mild folliculitis, furunculosis and atopic dermatitis to severe cases of necrotizing fasciitis.[1-3] Prolonged and ineffectively treated SSTIs may lead to endocartitis, osteomyelitis, meningitis, brain abscesses and pneumonia.[4,5] Mild SSTIs are treated with oral antimicrobial agents but complicated SSTIs lead to hospitalization, surgical intervention and treatment with intravenous antibiotics.[6,7]

The SENTRY antibiotic surveillance program reported that *Staphylococcus aureus* was the most frequently isolated pathogen isolated from SSTIs in North America, Latin America and Europe.[8] SSTIs reported in the emergency departments in the US increased from 1.2 million visits in 1993 to 3.4 million visits in 2005. This increase co-incided with the emergence of community-associated methicillin resistant *S. aureus*. Although no direct correlation can be made, this theory is supported by the fact that physicians started treating SSTIs from 2001 with antibiotics active against community-associated MRSA, namely trimethoprim-sulfamethoxazole and clindamycin.[9] The occurrence of antibiotic resistance among strains of *S. aureus* causing SSTIs has been reported by numerous researchers worldwide[10-12] and emphasizes the need for the development of alternative and more effective antimicrobial agents.

Optical imaging and bioluminescent reporter systems have in recent years advanced to a point where researchers can monitor bacterial infections in the same animal noninvasively and in real-time over multiple time-points.[13-17] Numerous studies have shown a strong correlation between the bioluminescence flux measurements generated from these genetically engineered bacteria and viable cell numbers in tissue homogenates.[14-17,18-20] Bioluminescent imaging (BLI) has been used for studying skin infections and the treatment of these infections with antibiotics and photodynamic therapy.[18,21,22]

Nisin F is a natural nisin variant with *in vitro* antimicrobial activity towards clinical strains of *S. aureus*.[23] Previously, we demonstrated that nisin F may play a protective role against *S. aureus* infections in the respiratory tract of Wistar rats.[24] The aim of this study is to investigate the antimicrobial effect of nisin F against *S. aureus* Xen 36, injected subcutaneously, in C57BL/6 mice using BLI.

Materials and Methods

Bacterial strains and culture conditions

Staphylococcus aureus Xen 36 (Caliper Life Sciences, Hopkinton, MA) has a stable copy of the modified *Photorhabdus luminescence luxABCDE* operon at a single integration site on a native plasmid.[15] The parental strain was *S. aureus* ATCC 49525, a clinical strain isolated from a bacteremia patient. *S. aureus* Xen 36 was cultured in brain heart infusion (BHI) (Biolab, Biolab Diagnostics, Midrand, South Africa) at 37°C. *Lactococcus lactis* subsp. *lactis* F10 was cultured in De Man Rogosa Sharpe (MRS) broth (Biolab) at 30°C.

In vitro *studies*

Nisin F, produced by *L. lactis* subsp. *lactis* F10,[23] was semipurified by ammonium sulphate precipitation and dialysis according to the method described by Sambrook *et al*.[25] The peptide was concentrated by freeze-drying in ampules. Antimicrobial activity was determined by using the agar-spot test method and expressed as arbitrary units (AU) per millilitre. One AU is the reciprocal of the highest serial two-fold dilution showing a clear zone of inhibition of the indicator strain.[26] The indicator strain was an 18 h-old culture of *S. aureus* Xen 36 (10^6 cfu/ml^1), embedded in BHI soft agar (1%).

Animal model

Approval for the experiments was obtained from the Animal Ethics Committee of Stellenbosch University (ethics reference number 200801024). C57BL/6 mice weighing between 20 and 25 g were divided into four groups (six per group). The hair on the back of each mouse was removed with Veet Lotion Hair Remover (Reckitt Benckiser, Elandsfontein, South Africa). The mice in group 1 were infected with *S. aureus* Xen 36 and treated with nisin F. Group 2 mice were infected with *S. aureus* Xen 36 and treated with sterile physiological saline. Group 3 mice were treated with nisin F, but not infected with *S. aureus* Xen 36. Group 4 mice were not infected and not treated. The mice were housed in plastic cages in animal rooms with constant environmental conditions and fed a standard rodent diet. The immune systems of the mice in all four groups were suppressed by adding deksamethasone to their drinking water (2.5 mg/L). Mice in group 1 and 2 were infected on day 1 by injecting 10 µl of

S. aureus Xen 36 (4 x 10^6 cfu) beneath the skin of each animal. Treatment took place on day 2 and 3 and consisted of 10 µl nisin F (256 AU) for groups 1 and 3 and 10 µl sterile physiological saline for group 2, injected subcutaneously.

On day 6 all the mice were euthanased by an overdose with pentobarbitone sodium (Centaur Labs, Bayer Animal Health Isando, South Africa) administered intraperitoneally. The chest cavity was opened and blood samples collected by cardiac puncture of the right ventricle.

In vivo imaging

Mice, while under 2% isoflurane gas anesthesia, were imaged each day for a maximum of 5 min using a CCD camera (IVIS® 100 Imaging System, Caliper Life Sciences). Living Image software (Caliper Life Sciences) was used to detect and quantify total photon emission (number of photons/s/cm^2) from defined regions of interest (ROI) within each image.

Histological studies

Sections of the skins of three rats from each group were aseptically removed, fixed in 4% (v/v) formaldehyde (PBS) for 24 h at 25ºC, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). The samples were processed and analysed at Pathcare Veterinary Pathologists (Pathcare, Dietrich, Voigt, Mia and Partners, Goodwood, South Africa).

Results and discussion

Research on the potential of nisin in treating skin and subcutaneous skin infections is very limited. Valenta *et al.* concluded that nisin is stable and antimicrobial against *S. aureus* in different topical formulations in *in vitro* studies.[27] The only study that investigated the *in vivo* effect of nisin, or any other bacteriocin, administered subcutaneously was performed by Ghiselli *et al*. Grafts were implanted subcutaneously and infected with *Staphylococcus epidermis*. Grafts soaked in nisin before implantation had lower infection counts (6.2 x 10^3±1.3 x 10^3 cfu) than the grafts not treated (4.8 x 10^7±2.0 x 10^6 cfu).[28]

BLI was used in this study to determine whether nisin F injected subcutaneously protected mice against deep dermal staphylococcal infection. Histology was performed to detect any pathology and verify data collected from BLI.

The total photon emissions from infected mice treated with nisin F (group 1) and saline (group 2), respectively, increased by 0.5 log 24 h after infection with *S. aureus* Xen 36 (Figures 1 and 2). Abscesses underneath the skin were also visible after 24 h in mice from both groups. At 24 h and 48 h either nisin F (group 1) or sterile physiological saline (group 2) was administered subcutaneously. No significant difference could be detected between group 1 and group 2 throughout the duration of the trail (Figures 1 and 2). Nisin F had therefore no antimicrobial activity against *S. aureus* Xen 36 *in vivo*. Bunce *et al.* reported that the degree of cutaneous infections caused by *S. aureus* varies from mouse to mouse. The onset of lesions also differed between the mice. Both these observations are also seen among human cutaneous staphylococcal infections.[29] This can be a possible explanation for the high standard deviation in the bioluminescent flux measurements in the infected groups.

Subacute active necrotic dermatitis and cellulitis were detected in the mice from group 1 (infected and treated with nisin F). Multifocal areas of necrosis with complete loss of cellular detail were detected in the dermal stromas and deeper subcuticular tissues. These areas were associated with a high infiltration of polymorphonuclear cells, as well as lymphocytes and fibroblasts in the surrounding tissues. Occasional hair fragments were also visible (Figure 3a). Subacute to active dermatitis were detected in the mice from group 2 (infected and treated with saline). Multifocal areas of necrosis with complete loss of cellular detail were also detected in the dermal stromas of the mice in group 2. Small hair fragments were also visible. However, a reduction in the number of polymorphonuclear cells in the lesions of mice treated with saline was observed (Figure 3b). The innate immune system of mice treated with nisin may have been more actively combating the infection since they had a higher polymorphonuclear cell density. The other possible explanation is that the administration of nisin could have modulated the innate immune system.

The total photon emissions from mice not infected and treated with nisin F (group 3) and mice not infected and not treated (group 4) were significantly lower than the total photon emissons from the infected groups (group 1 and 2), as expected. The total photon emissions from group 3 were slightly higher than the total photon emissions from group 4 (Figures 1 and 2). This is an interesting phenomenon since the only variable between the two groups was the administration of nisin which does not bioluminesce.

The histopathology of the skin of the mice in group 3 (not infected and treated with nisin F) ranged from no histological changes to focal acute dermatitis. Focal areas of oedema with mild infiltration of polymorphonulear cells as well as a few macrophages were detected in the dermal stroma of the mouse with focal acute dermatitis and the mouse with mild focal dermal oedema. The cytoplasma of the macrophages were discoloured in the latter mouse, probably due to pigment in the protein product (Figure 3c). Nisin F may thus modulate the innate immune system when injected subcutaneously. No histological changes were observed in the mice from group 4 (not infected and not treated) (Figure 3d).

Future research is needed to evaluate nisin F as a topical antimicrobial in superficial skin infections. Kugelberg *et al.* have established *S. aureus*-related superficial skin infection models by removing the epidermis of mice skin with tape stripping.[30] The application of nisin F as a topical agent will not only determine if it is more effective as an antimicrobial agent, but will also investigate whether the innate immune system will be aggravated. The effect that nisin and other lantibiotics have on the immune system in murine models is an important topic since research on the medical applications of lantibiotics is increasing. This is especially relevant when applying nisin as an antimicrobial agent for skin infections since atopic dermatitis, although not yet defined as an auto-immune disease, is initiated and maintained by the actions of inflammatory cells such as cytokines, chemokines and T-cells.[31]

In conclusion we have reported that the subcutaneous administration of nisin F had no effect on deep dermal staphylococcal infections. Furthermore, the administration of nisin F subcutaneously may have modulated the innate immune system.

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References

1. Carré N, Sillam F, Dabas JP *et al. Staphylococcus aureus* carrying Panton-Valentine leukocidin genes nasal colonization and skin infection: screening in case of outbreak in a school environment. *Med Mal Infect* 2008; **38:** 483-488.

2. Leyden JJ, Marples RR, Kligman AM. *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br J Dermatol* 1974; **90:** 525-530.

3. Young LM, Price CS. Community-acquired methicillin-resistant *Staphylococcus aureus* emerging as an important cause of necrotizing fasciitis. *Surg Infect (Larchmt)* 2008; **9:** 469-474.

4. Lo BM, Erwin EA. Missed epidural brains abscess after furunculosis. *Am J Emerg Med* 2008; **26:** 522.

5. Stevens DL, Smith LG, Bruss JB *et al.* Randomized comparison of linezolid (PNU-100766) versus oxacillin-dicloxacillin for treatment of complicated skin and soft tissue infections. *Antimicrob Agents Chemother* 2000; **44:** 3408-3413.

6. Nichols RL, Florman S. Clinical presentations of soft-tissue infections and surgical site infections. *Clin Infect Dis* 2001; **33:** 84-93.

7. Wilson SE. Clinical trial results with linezolid, an oxazolidinone, in the treatment of soft tissue and postoperative Gram-positive infections. *Surg Infect (Larchmt)* 2001; **2:** 25-35.

8. Moet GJ, Jones RN, Biedenbach DJ *et al.* Contemporary causes of skin and soft tissue infections in North America, Latin America, and Europe: report from the SENTRY Antimicrobial Surveillance Program (1998-2004). *Diagn Microbiol Infect Dis* 2007; **57:** 7-13.

9. Pallin DJ, Egan DJ, Pelletier AJ *et al.* Increased US emergency department visits for skin and soft tissue infections, and changes in antibiotic choices, during the emergence of community-associated methicillin-resistant *Staphylococococcus aureus*. *Ann Emerg Med* 2008; **51:** 291-298.

10. Awad SS, Elhabash SI, Lee L *et al.* Increasing incidence of methicillin-resistant *Staphylococcus aureus* skin and soft-tissue infections: reconsideration of empiric antimicrobial therapy. *Am J Surg* 2007; **194:** 606-610.

11. Bell JM, Turnidge JD SENTRY APAC. High prevalence of oxacillin-resistant *Staphylococcus aureus* isolates from hospitalized patients in Asia-Pacific and South Africa: results from SENTRY antimicrobial surveillance program, 1998-1999. *Antimicrobial Agents Chemother* 2002; **46:** 879-881.

12. Kim ES, Song JS, Lee HJ *et al.* A survey of community-associated methicillin-resistant *Staphylococcus aureus* in Korea. *J Antimicrob Chemother* 2007; **60:** 1108-1114.

13. Contag CH, Contag PR, Mullins JI *et al.* Photonic detection of bacterial pathogens in living hosts. *Mol Microbiol* 1995; **18:** 593-603.

14. Francis KP, Joh D, Bellinger-Kawahara C *et al.* Monitoring bioluminescent *Staphylococcus aureus* infections in living mice using a novel *luxABCDE* construct. *Infect Immun* 2000; **68:** 3594-3600.

15. Francis KP, Yu J, Bellinger-Kawahara C *et al.* Visualizing pneumococcal infections in the lungs of live mice using bioluminescent *Streptococcus pneumoniae* transformed with a novel Gram-positive lux transposon. *Infect Immun* 2001; **69:** 3350-3358.

16. Kadurugamuwa JL, Sin L, Albert E *et al.* Direct continuous method for monitoring biofilm infection in a mouse model. *Infect Immun* 2003; **71:** 882-890.

17. Mortin LI, Li T, van Praagh AD *et al.* Rapid bactericidal activity of daptomycin against methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* peritonitis in mice as measured with bioluminescent bacteria. *Antimicrob Agents Chemother* 2007; **51:** 1787-1794.

18. Hamblin MR, Zahra T, Contag CH *et al.* Optical monitoring and treatment of potentially lethal wound infections *in vivo*. *J Infect Dis* 2003; **187:** 1717-1725.

19. Jawhara S, Mordon S. *In vivo* imaging of bioluminescent *Escherichia coli* in a cutaneous wound infection model for evaluation of an antibiotic therapy. *Antimicrob Agents Chemother* 2004; **48:** 3436-3441.

20. Rocchetta HL, Boylan CJ, Foley JW *et al.* Validation of a noninvasive, real-time imaging technology using bioluminescent *Escherichia coli* in the neutropenic mouse thigh model of infection. *Antimicrob Agents Chemother* 2001; **45:** 129-137.

21. Burkatovskaya M, Tegos GP, Swietlik E *et al.* Use of chitosan bandage to prevent fatal infections developing from highly contaminated wounds in mice. *Biomaterials* 2006; **27:** 4157-4164.

22. Hamblin MR, O'Donnell DA, Murthy N *et al.* Rapid control of wound infections by targeted photodynamic therapy monitored by *in vivo* bioluminescence imaging. *Photochem Photobiol* 2002; **75:** 51-57.

23. de Kwaadsteniet M, Ten Doeschate K, Dicks LM. Characterization of the structural gene encoding nisin F, a new lantibiotic produced by a *Lactococcus lactis* subsp. *lactis* isolate from freshwater fish (*Clarius gariepinus*). *Appl Environ Microbiol* 2008; **74:** 547-549.

24. de Kwaadsteniet M, Ten Doeschate K, Dicks LM. Nisin F in the treatment of respiratory tract infections caused by *Staphylococcus aureus. Lett Appl Microbiol* 2009; **48:** 65-70.

25. Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: A laboratory manual, 2 nd Ed.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.

26. van Reenen CA, Dicks LM, Chikindas ML. Isolation, purification and partial characterization of plantaricin 423, a bacteriocin produced by *Lactobacillus plantarum. J Appl Microbiol* 1998; **84:** 1131-1137.

27. Valenta C, Bernkop-Schnürch A, Rigler HP. The antistaphylococcal effect of nisin in a suitable vehicle: a potential therapy for atopic dermatitis in man. *J Pharm Pharmacol* 1996; **48:** 988-991.

28. Ghiselli R, Giacometti A, Cirioni O *et al.* RNAIII-inhibiting peptide and/or nisin inhibit experimental vascular graft infection with methicillin-susceptible and methicillin-resistant *Staphylococcus epidermis*. *Eur J Vasc Endovasc Surg* 2004; **27:** 603-607.

29. Bunce C, Wheeler L, Reed G *et al.* Murine model of cutaneous infection with gram-positive cocci. *Infect Immun* 1992; **60:** 2636-2640.

30. Kugelberg E, Norström T, Petersen TK *et al*. Establishment of a superficial skin infection model in mice by using *Staphylococcus aureus* and *Streptococcus pyogenes*. *Antimicrob Agents Chemother* 2005; **49:** 3435-3441.

31. Chan LS. Atopic dermatitis in 2008. *Curr Dir Autoimmun.* 2008; **10:** 76-118.

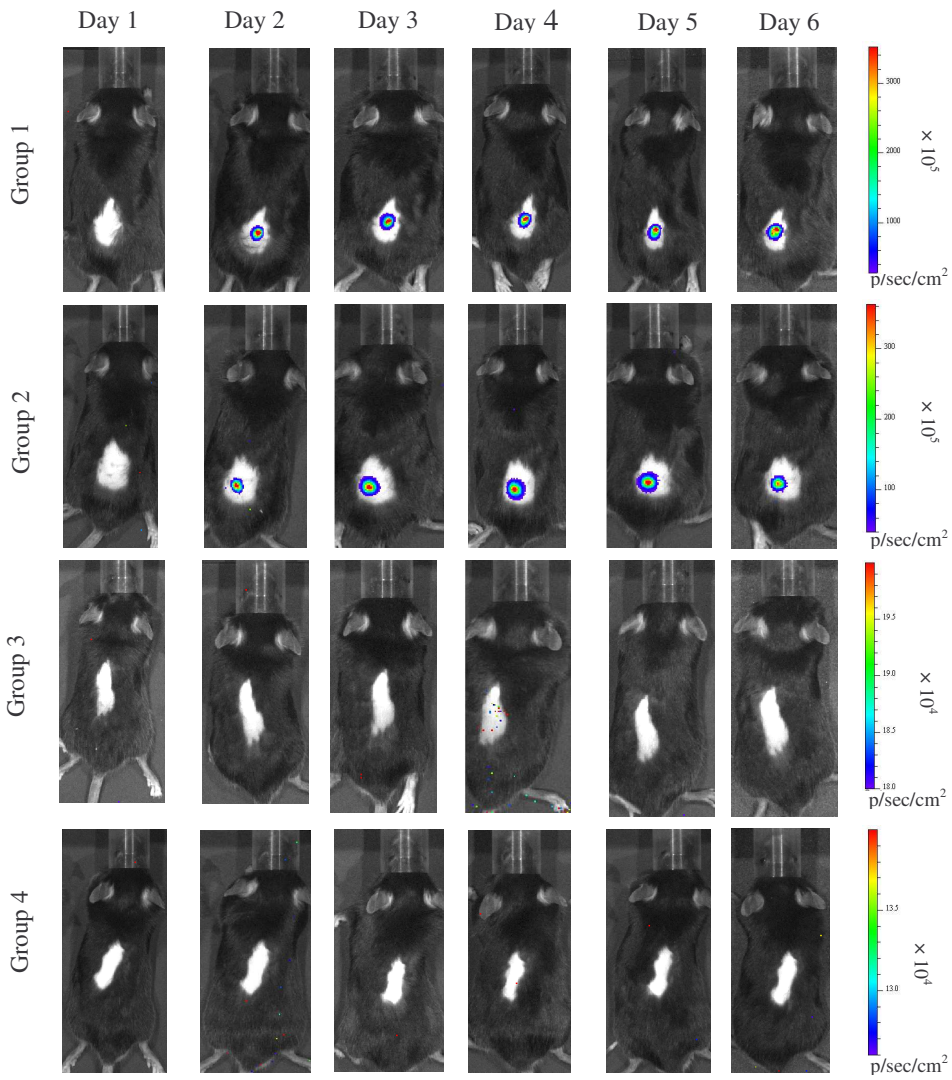

Figure 1. A representative mouse from each group depicts a time series of photon detection (photons per second per square centimeter) from metabolically active *S. aureus* Xen 36. Group1: infected and treated with nisin F, group 2: infected and treated with saline, group 3: not infected and treated with nisin F and group 4: not infected and not treated.

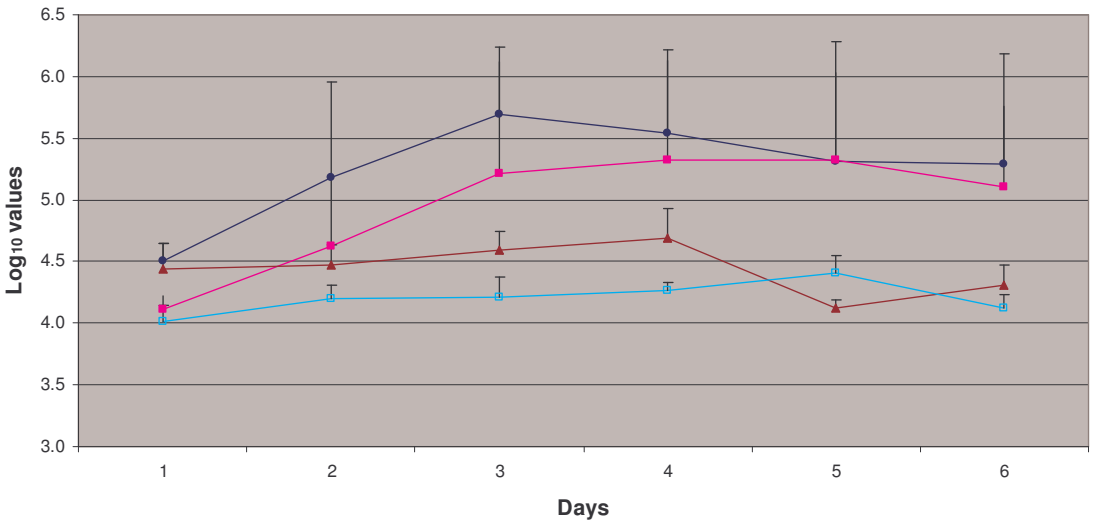

Figure 2. Photon emissions (photons/s/cm^2/sr) from defined ROI within selected images of the mice in each group. Group1 (-●-): infected and treated with nisin F, group 2 (-■-): infected and treated with saline, group 3 (-▲-): not infected and treated with nisin F and group 4 (-□-): not infected and not treated.

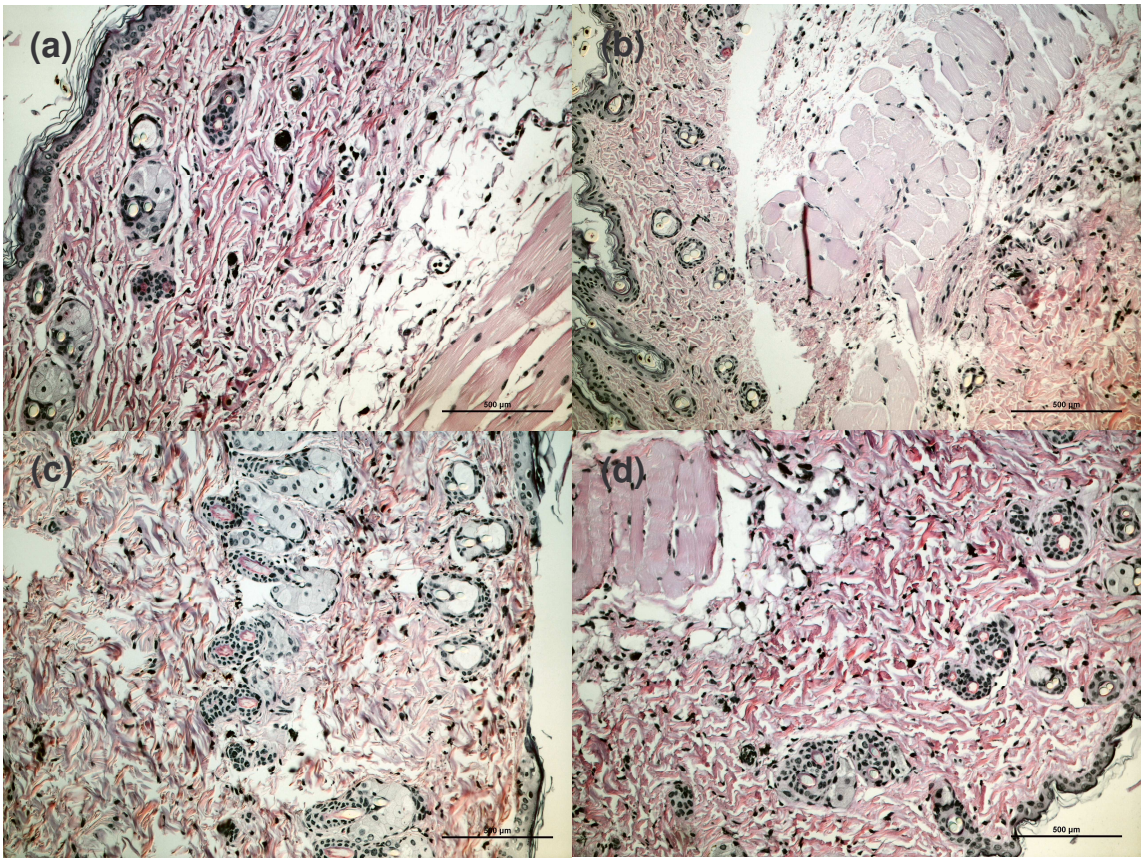

Figure 3. Histology images of skin tissue from (a) an infected and treated with nisin F mouse, (b) an infected and treated with saline mouse, (c) a not infected and treated with nisin F mouse and (d) a not infected and not treated mouse.

Chapter 6

General discussion and conclusions

Traditionally, bacteriocins have been used in food preservation. Recently, the potential application of bacteriocins as an alternative to antibiotics has been explored. This study entailed the isolation and characterization of a lantibiotic, nisin F, proven to be active against clinical *Staphylococcus aureus* strains *in vitro* and the potential antimicrobial activity *in vivo* in murine models.

Lactococcus lactis subsp. *lactis* F10, isolated from fresh water catfish, *Clarias gariepinus*, produces a lantibiotic, nisin F, active against two clinical *Staphylococcus aureus* strains, *Staphylococcus carnosus* LMG 13556, *Lactobacillus curvatus* LMG 13553, *Lactobacillus plantarum* LMG 13556 and *Lactobacillus reuteri* LMG 13557. Plasmid curing with 30 µg ml^{-1} ethidium bromide revealed that the genes encoding the production, immunity and secretion of the peptide are located on a 24-kb plasmid. Sequencing of the structural gene revealed that nisin F relates to nisin Z by having asparagine at position 27 and not histidine as recorded for nisin A. However, nisin F differs from nisin Z by having valine at position 30 instead of isoleucine and is in this respect similar to nisin Q. Nisin F differs from nisin Q by having alanine at position 15 and not valine, and methionine at position 21 instead of leucine. Nisin Q was also isolated from a water source, namely a river in Japan (Zendo *et al.,* 2003).

Nisin is active against Gram-positive pathogenic bacteria of fish (Elotmani and Assobhei, 2004) and modulates the immune system of turbot (Villamil *et al.,* 2003). Both of these mechanisms can protect fish against disease and may explain the presence of nisin-producing *L. lactis* subsp. *lactis* strains in freshwater catfish and in rivers of Japan (de Kwaadsteniet *et al.,* 2008; Zendo *et al.,* 2003). The interest in using live lactic acid bacteria as probiotics in fish farming further supports this theory (Gatesoupe, 2008). Nisin F is regarded as a new variant of the nisin family of lantibiotics and is referred to as nisin F.

Nisin F proved very effective *in vitro* against clinical *S. aureus* strains isolated from sinusitis patients (de Kwaadsteniet *et al.,* 2008). Nisin F, in combination with either lysozyme or lactoferrin, has a synergistic effect *in vitro* (de Kwaadsteniet *et al.,* 2009). Lysozyme and lactoferrin are the two most abundantly secreted antimicrobial proteins in airway secretions of humans (Brogan *et al*. 1975; Harbitz *et al*. 1984; Wilson 2005). These *in vitro* results prompted an investigation towards the antimicrobial potential of nisin F against *S. aureus* infections in the respiratory tract.

The antimicrobial effect of nisin F was tested *in vivo* in non-immunosuppressed and immunosuppressed Wistar rats. Nisin F or physiological sterile saline (control) was applied intranasally to *S. aureus* infected rats. Histology of non-immunosuppressed rats infected with *S. aureus* K revealed that the rats were healthy. The immune system of rats must therefore be compromised for studying *S. aureus* respiratory tract infections as Kruzsweska *et al.* (2004) concluded during a previous study with cotton rats.

Bacterial infected, immunosuppressed and saline treated rats had the following symptoms of respiratory tract infection. Histology detected pneumoniae in the lungs, i.e. obliterated alveoli probably due to the infiltration of macrophages, granulocytes and lymphocytes, and the proliferation of fibroblasts and alveolar epithelial cells; and mucosal hyperplasia in the trachea. Mucosal hyperplasia occurs usually due to an increased mucus production, a characteristic of staphylococcal infections (Dohrman *et al.* 1998; Basbaum *et al*. 1999). Necrotic lesions were also visible on the lungs and blood contamination was detected in one of the rats, indicative of damage lung tissue through which the pathogen migrates into the blood stream. None of the above mentioned symptoms were detected in the infected, immunosuppressed and nisin F-treated rats. This indicated that nisin F protected the respiratory tract of the rats. No bacteremia, necrotic lesions, mucosal hyperplasia or pneumoniae were recorded.

Kokai-Kun *et al.* (2003) applied nisin intranasally, in a petroleum-based cream formulation, to cotton rats infected with *S. aureus*. Nisin was unable to eradicate *S. aureus* from the nares of the rats although it had antistaphylococcal activity *in vitro*. Researchers concluded that nisin was either inactivated or absorbed in the nares since no antistaphylococcal activity could be detected from the nares of the nisin-treated rats. This study focused on the effect of nisin on the colonization of *S. aureus* in the respiratory tract of rats and hence did not include clinical parameters such as bacteremia or histopathology. The other difference between the Kokai-Kun *et al.* and our study is that nisin was applied in a cream formulation in their study and during our investigation nisin was applied in a water-based formula. Kruszewska *et al.* (2004) also investigated the effect of a lantibiotic, mersacidin, on the colonization of the nares of immunosuppressed mice with *S. aureus*. Mersacidin was successful in eradicating *S. aureus* from the nares. Interleukin 1β was not detected in mersacidin-treated mice. The missing immune reaction is also indicative on the curative effect of mersacidin.

The safety of administering nisin F intranasally had to be evaluated. The joint FDA/WHO Expert Committee on Food additives (JECFA) have only declared nisin A and Z to be safe for human consumption (WHO, 1969). Nisin F was shown to have no known adverse effects in the respiratory tract of Wistar male rats (de Kwaadsteniet *et al.,* 2009). The intranasal administration of nisin F had no effect on weight gain or on the amount of food and water consumed. The lungs, trachea and bronchi of rats (non-immunosuppressed and immunosuppressed) treated with nisin F revealed no histopathology and was similar when compared to the control groups (saline treated rats). Nisin F had no hemolytic activity *in vivo* since the red blood indices between the nisin-treated and control groups were similar (non-immunosuppressed and immunosuppressed rats). Nisin F also had no effect on white blood cells, lymphocytes and neutrophils production since the levels between the nisin-treated and control groups (non-immunosuppressed and immunosuppressed rats) were comparable. Concluded from these findings, the intranasal administration of nisin F is safe and nontoxic. This endorses the use of nisin F in the treatment of respiratory tract infections.

Newly developed methodology, bioluminescent imaging (BLI), was used to monitor the effect of nisin F against *S. aureus*-related infections in real-time, noninvasively in live animals. Nisin F is active against *S. aureus* Xen 36, a strain engineered with the *luxABCDE* operon integrated at a single site on a native plasmid. Non-immunosuppressed and immunosuppressed C57BL/6 mice were infected with *S. aureus* Xen 36 intranasally and imaged in the IVIS 100 System for bioluminescence. The immune system of the mice was suppressed with either deksamethasone or 3 Gy X-ray irradiation. Unfortunately, the bioluminescence detected in the mice was not consistent and thus not reproducible. The parental strain of *S. aureus* Xen 36 was *S. aureus* ATCC 49525, a clinical strain isolated from a bacteremia patient. It is possible that *S. aureus* Xen 36 were unable to colonize the respiratory tract of the mice since it was not originally a nasal isolate, unlike *S. aureus* K used in the Wistar rat model.

The advantage of studying skin and subcutaneous skin infections with BLI technology is that the infections is on the surface of the C57BL/6 mice since photon intensity decreases ten fold with each centimeter of tissue depth (Sadikot and Blackwell, 2005). The bioluminescent bacteria, *S. aureus* Xen 36, was easily detected and monitored. *S. aureus* Xen 36 injected subcutaneously also successfully infected the mice since this strain was originally isolated from a bacteremia patient, as already mentioned. In this experiment C57BL/6 mice were grouped into four groups; infected nisin-treated mice, infected saline treated mice,

noninfected nisin-treated mice and noninfected and nontreated mice. The immune system of all the mice was suppressed by adding deksamethasone to their drinking water throughout the duration of the trail. Mice were treated with either nisin F or sterile physiological water 24 h and 48 h after infection with subcutaneously injected *S. aureus* Xen 36.

The bioluminescence flux measurements (total photon emmisions) between the infected, nisin-treated mice and the infected, saline treated mice did not differ significantly. Histology of skin tissues revealed that both groups of mice suffered from subacute to active dermatitis with multifocal areas of necrosis in the dermal stromas and deeper subcuticular tissues. The only difference was the higher number of polymorphonuclear cells detected in the lesions of the infected, nisin-treated mice. Nisin F was therefore ineffective in combating subcutaneous staphylococcal infection. The bioluminescence flux measurements of the non-infected, nisin-treated mice and non-infected and non-treated mice were significantly lower than the measurements of the infected mice groups, as expected. Histology of skin tissues of noninfected, nisin-treated mice detected symptoms ranging from no changes to focal acute dermatitis. Focal areas of oedema with mild infiltration of polymorphonulear cells as well as a few mast cells were detected in the dermal stroma of the mouse with focal acute dermatitis and the mouse with mild focal oedema. No histological changes were observed in the skin tissues of noninfected and nontreated mice.

Mice treated with nisin (both the infected and noninfected groups) had a higher number of polymorphonuclear cells in the dermal stromas of the skin tissue. This indicates that nisin F may have stimulated the immune system when injected subcutaneously. These are only preliminary results and must be further investigated since the interest of nisin and other lantibiotics as medical antimicrobials are growing. Atopic dermatitis is initiated and maintained by the actions of inflammatory cells such as cytokines, chemokines and T-cells (Chan, 2008). Great care must therefore be taken since subcutaneously injected nisin, if acting as an immune modulator, could worsen atopic dermatitis.

Future research will include investigating the effect of nisin F as a topical antimicrobial on superficial skin infections. The noninvasive application of nisin F as a topical agent will not only determine if it is more effective as an antimicrobial but will also investigate whether the innate immune system will also be aggravated. Nisin F can also be incorporated in chitosan acetate bandages, already used in wound dressing, to investigate whether it will increase the antimicrobial efficiency. Burkatovskaya *et al.* (2006) used BLI to investigate whether

chitosan acetate bandages prevented bioluminescent *S. aureus, Proteus mirabilis* and *Pseudomonas aureuginosa* from infecting wounds.

To conclude, nisin F is a new natural nisin variant produced by *L. lactis* subsp. *lactis*, isolated from the freshwater catfish, *Clarias gariepinus*. Nisin F has shown antimicrobial activity against clinical *S. aureus* strains *in vitro* and the antimicrobial properties were further tested *in vivo* using murine models. Nisin F, applied intranasally, protected the respiratory tract of rats against *S. aureus* K. Nisin F applied intranasally proved to have no adverse effects on the respiratory tract and hence safe for use. Subcutaneously injected nisin F was however unable to protect mice against deep dermal staphylococcal infection, *S. aureus* Xen 36 being the causative agent. Also, the safety of subcutaneously injected nisin F is questionable since it may have modulated the immune system of the mice. Nisin F treatments had different outcomes, depending on the site of infection and the route of administration. Nisin F as an alternative treatment for *S. aureus* related infections have displayed positive and negative results. This area of research has only recently developed and these preliminary results need to be verified by more in-depth research since animals and humans are complex systems.

References

Basbaum, C., H. Lemjabbar, M. Longphre, D. Li, E. Gensch, and N. McNamara. 1999. Control of mucin transcription by diverse injury-induced signaling pathways. Am. J. Respir. Crit. Care Med. **160:**S44-S48.

Brogan, T. D., H. C. Ryley, L. Neale, and J. Yassa. 1975. Soluble proteins of bronchopulmonary secretions from patients with cystic fibrosis, asthma, and bronchitis. Thorax **30:**72-79.

Burkatovskaya, M., G. P. Tegos, E. Swietlik, T. N. Demidova, A. Castano, and M. R. Hamblin. 2006. Use of chitosan bandage to prevent fatal infections developing from highly contaminated wounds in mice. Biomaterials **27:**4157-4164.

Chan, L. S. 2008. Atopic dermatitis in 2008. Curr. Dir. Autoimmun. **10:**76-118.

de Kwaadsteniet, M., K. Ten Doeschate, and L. M. Dicks. 2008. Characterization of the structural gene encoding nisin F, a new lantibiotic produced by a *Lactococcus lactis* subsp. *lactis* isolate from freshwater fish (*Clarius gariepinus*). Appl. Environ. Microbiol. **74:**547-549.

de Kwaadsteniet, M., K. Ten Doeschate, L. M. Dicks. 2009. Nisin F in the treatment of respiratory tract infections caused by *Staphylococcus aureus.* Lett. Appl. Microbiol **48:**65-70.

Dohrman, A., S. Miyata, M. Gallup, J. D. Li, C. Chapelin, A. Coste, E. Escudier, J. Nadel, and C. Basbaum. 1998. Mucin gene (*MUC 2* and *MUC 5AC*) upregulation by Gram-positive and Gram-negative bacteria. Biochim. Biophys. Acta. **1406:**251-259.

Elotmani, F. and O. Assobhei. 2004. *In vitro* inhibition of microbial flora of fish by nisin and lactoperoxidase system. Lett. Appl. Microbiol. **38:**60-65.

Gatesoupe, F. J. 2008. Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments. J. Mol. Microbiol. Biotechnol. **14:**107-114.

Harbitz, O., A. O. Jenssen, and O. Smidsrød. 1984. Lysozyme and lactoferrin in sputum from patients with chronic obstructive lung disease. Eur. J. Respir. Dis. **65:**512-520.

Kokai-Kun, J. F., S. M. Walsh, T. Chanturiya, and J. J. Mond. 2003. Lysostaphin cream eradicates *Staphylococcus aureus* nasal colonization in a cotton rat model. Antimicrob. Agents Chemother. **47:**1589-1597.

Kruszewska, D., H. G. Sahl, G. Bierbaum, U. Pag, S. O. Hynes, and A. Ljungh. 2004. Mersacidin eradicates methicillin-resistant *Staphylococcus aureus* (MRSA) in a mouse rhinitis model. J. Antimicrob. Chemother. **54:**648-653.

Sadikot, R. T., and T. S. Blackwell. 2005. Bioluminescence imaging. Proc. Am. Thorac. Soc. **2:**537-540.

Villamil, L., A. Figueras, and B. Novoa. 2003. Immunomodulatory effects of nisin in turbot (*Scophthalmus maximus L.*). Fish Shellfish Immunol. **14:**157-169.

WHO Expert Committee on Biological Standardization. 1969. Twenty-second report, WHO Technical Report Series No. 444.

Wilson, M. 2005. Microbial Inhabitants of Humans. Their Ecology and Role in Health. Cambridge University Press, Cambridge, UK.

Zendo, T., M. Fukao, K. Ueda, T. Higuchi, J. Nakayama, and K. Sonomoto. 2003. Identification of the lantibiotic nisin Q, a new natural nisin variant produced by *Lactococcus lactis* 61-14 isolated from a river in Japan. Biosci. Biotechnol. Biochem. **67:**1616-1619.